

Characterization and Anaerobic Digestion Analysis of Ethanol Process Samples

By

Pinnacle Biotechnologies Inc.



National Renewable Energy Laboratory

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July 1, 1998

Nick Nagle
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Dear Nick,

This letter report and the accompanying invoice serves as the conclusion of activities under NREL procurement P.O. #160809. Sample characterization data were summarized in a previous letter report. Presently, the anaerobic fermentation data and conclusions are described.

The Anaerobic Fermentation Bioassay

It is important to note at the outset that the BMP assay may be useful in determining the potential level of bioconversion which may be possible for a test substrate. This assay may also give indications of a potential for a test substrate to cause inhibition of the anaerobic consortium which would limit or preclude conversion of the test substrate at least under anaerobic conditions. However, the BMP assay is always viewed as a rough cut analysis, with evaluation of continuous anaerobic digestion systems as a natural next step to provide better process data on rates and yields prior to engineering and costing commercial systems. The BMP assay may also be used to determine the effectiveness of treatments aimed at reducing sample toxicity or to improve the potential conversion rates and yields. Several important issues regarding the anaerobic fermentation studies (biochemical methane potential [BMP] assay) must be discussed prior to the interpretation of the data.

The Anaerobic Culture. A robust, diverse, anaerobic culture from a reliable, defined source is important to establishing the best fermentation analysis data. PINNACLE uses anaerobic cultures from anaerobic digesters at local municipal sewage treatment plants as assay and starter cultures as these cultures; 1) see a diverse mixture of organic wastes and therefore the microbial populations are diverse in biodegradative capabilities, 2) receive substantial macro and micro-nutrients and therefore are not operating under limiting or inhibitory conditions, and 3) are readily available and may be further obtained in large quantities for starting large scale applied systems once sufficient testing data is obtained.

The quantity of test culture used in the anaerobic fermentation assays is maximized to ensure rapid biodegradative results and to reduce the potential negative effects of dilution on the activity



of the culture.

Negative Control. A set of three negative controls were used during anaerobic fermentation studies to account for biogas production due to intrinsic organic matter contained in the anaerobic culture. It should be noted that any active culture used in fermentation tests will produce biogas from intrinsic organic matter unless the culture is first "washed" to remove this material first. For anaerobic fermentation studies, culture washing is detrimental to culture viability due to the potential to introduce oxygen or removal of complex macro and micro-nutrients. Without removing the intrinsic organics contained in the anaerobic culture, it is possible that an added test sample will negatively or positively affect the conversion of the intrinsic culture organics and therefore the background biogas production.

Positive Control. Generally, a positive control is selected which is similar to the composition of the test samples and which can serve as a check on the biodegradative capacity of the anaerobic culture used. The positive control is prepared at similar pH and organic loading to the test samples.

Anaerobic Fermentation Studies

Test Samples. Test sample characterizations were described in a previous letter report and indicated that samples MTX 7F, TiO₂, and the Control Hydrolyzate were comparable in mass percent volatile solids (organic content) while sample BF 772014 was nearly 50% more dilute. The pH of all test samples were considerably below pH 7.0 and required adjustment with potassium hydroxide prior to fermentation studies. The analysis of chemical oxygen demand (COD), a measure of oxidizable carbon in the sample, indicated samples TiO₂ and the Control Hydrolyzate were similar and the highest of the samples while BF 772014 was the lowest.

Positive Control. For the positive control, a solution of protein hydrolyzate (BactoPeptone, Difco) was used. The use of a protein hydrolyzate sample was envisioned to be relatively close to the composition of the ethanol hydrolyzate samples. The mass percent volatile solids and COD values for the positive control sample were only slightly greater than samples TiO₂ and the Control Hydrolyzate.

Pre-Incubation and Startup. Anaerobic fermentation assays were initiated following incubation of the assay bottles for almost four days in order to reduce the background biogas production derived from the intrinsic organics in the anaerobic culture. A single volumetric loading was used (5%) which resulted in varying organic loadings for the different test samples from 1.41 to 2.87 grams of COD per liter of culture due to their individual concentrations.



Results. Immediate and strong biogas production was determined for all test samples as detailed in Figure 1. All samples also demonstrated the majority of the biogas production, hence the sample organic conversion, was complete within 5 to 10 days. The overall level of anaerobic bioconversion for each test sample is shown in Figure 2 based on the individual sample COD loading. A theoretical yield of 350 mL of methane per gram of COD added represents 100% conversion (Owen and McCarty, 1964). Anaerobic conversion data is shown in Table 1, below for the test samples after 26 days of incubation.

Table 1. Anaerobic Fermentation Data and Final Analyses (26 d)

Assay	BF 772014	MTX 7F	TiO2	Control Hyd.	Bacto Peptone
COD Loading (gCOD/bottle)	0.141	0.174	0.279	0.272	0.287
Theoretical CH4 Yield (mL)	49.35	60.90	97.65	95.20	100.45
Actual CH4 Yield (mL)	36.07	75.16	35.39	76.93	83.01
% Anaerobic Conversion	73.09	123.42	36.24	80.81	82.64
Final Biogas Methane (%)	61.40	61.86	64.56	61.43	64.98
Final pH	7.23	7.22	7.24	7.24	7.36

In general, the data indicates that the positive control (BactoPeptone), the Control Hydrolyzate, and BF 772014 resulted in similar levels of bioconversion (70% to 80%). If these samples were to be further incubated to 90 days, the final level of anaerobic conversion based on COD loading would most likely range from 90% to 100% of the theoretical. This slow approach to near complete digestion during the extended incubation period (final 60 days of a 90 day test) represents the adaptation of the anaerobic culture to minor, less common organics in the test samples.

The results found for the positive control, the Control Hydrolyzate and BF 772014 are characteristic of organic wastes which are eminently biodegradable. Test sample TiO2 demonstrated limited biogas production indicating that organics in the sample were only partly biodegradable.

BMP data for NREL sample MTX 7F indicated greater than 100% conversion to the methane endproduct. This may be explained as either inaccurate COD analysis or active enzymes



contained in the sample which are effective in converting recalcitrant intrinsic organics (i.e., polymers) of the seed culture. Table 2. compares initial and final COD analysis for all four NREL test samples and validates the relative accuracy of the assay.

Table 2. Re-Evaluation of NREL Test Sample COD Values

Assay	BF 772014	MTX 7F	TiO2	Control Hyd.
Primary COD Assay (mg/L)	28,267	34,800	55,800	54,400
Secondary COD Assay (mg/L)	26,330	32,330	53,330	55,660
Difference (%)	-6.85	-7.10	-4.43	+2.32

As the accuracy of the test sample COD values are assured, the only plausibly explanation is sample MTX 7F contained active hydrolytic enzymes which served to hydrolyze recalcitrant organics contained in the starter culture. Methods to test this theory and determine the true nature of the anaerobic biodegradation potential for this sample may include a thermal treatment of the sample to inactivate enzymes followed by conducting another BMP assay. In addition, the test sample could be analyzed by standard method for hydrolyzing enzyme activity.

Conclusion

All samples tested demonstrated immediate and strong biogas production. None of the samples tested demonstrated toxicity to the anaerobic culture. The positive control demonstrated predicted effectiveness of the anaerobic starter culture. NREL samples BF 772014 and the Control Hydrolyzate demonstrated conversions similar to that of the positive control and may therefore be considered amenable to anaerobic treatment. NREL sample TiO2 demonstrated reduced conversion effectiveness which is likely due to some level of non-biodegradable organics in the sample. The excessive biogas production resulting in assays performed using NREL sample MTX 7F indicates that additional testing as described above is required to accurately predict the level of conversion possible.

While this data may be used to predict approximate fuel gas production which may result from treating large volumes of the respective organic steams using anaerobic digestion systems, in order to accurately engineer commercial-scale anaerobic systems, additional data from applied, longer-term operation of continuous anaerobic digestion systems should be obtained.



Nick Nagle
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Dear Nick,

The four NREL samples received from you were stored under refrigeration until being transferred by cooler to PINNACLE's Research, Development and Testing Center in Stanton, California for analysis and fermentation studies.

Rather than using Avecel as the positive control for these studies, a soluble substrate was used which more closely matches the NREL samples. The positive control substrate used was a Bacto Peptone solution at 4.5% w/v in distilled water. The NREL samples were analyzed on delivery PINNACLE's Testing Center. Total solids (%TS), volatile solids (%VS), and ash analyses were performed in triplicate. Analysis of sample pH were performed after a 2-point standardization of the combination pH probe.

Table 1. Sample Analysis Upon Receipt

Assay	BF 772014	MTX 7F	TiO2	Control Hyd.	Bacto Peptone
% Total Solids	2.73	4.49	5.56	5.20	5.67
% Volatile Solids	87.44	89.86	74.04	82.33	95.67
Mass % Volatile Solids	2.39	4.03	4.12	4.28	5.42
pH	5.39	4.93	5.24	5.36	7.08

As the NREL samples were considerably lower than the pH 7.0 necessary to perform the anaerobic digestibility analysis, they were adjusted to neutrality using a 5% w/v solution of KOH. A 50 mL aliquot of each sample was transferred to a small beaker. The sample was mixed using a magnetic stirrer and the pH monitored during KOH addition. The samples were then analyzed for Chemical Oxygen Demand (COD) using the HACH High Range Plus COD tube assay. All COD assays were performed in triplicate as detailed in Table 2.



Table 2. Sample pH Adjustment and COD Analysis

Assay	BF 772014	MTX 7F	TiO2	Control Hyd.
Initial pH	5.39	4.93	5.24	5.36
mL KOH Added	0.56	1.22	1.11	0.8
Dilution Factor	0.9889	0.9762	0.9783	0.9843
Final pH	7.12	7.08	7.13	7.14
COD (mg/L)	28,267	34,800	55,800	54,400

For comparison, the COD level of the Bacto Peptone positive control was 57,400 mg/L.

Anaerobic Digestibility Assays

The Biochemical Methane Potential (BMP) assay was used to address the biodegradability or toxicity of the NREL samples. The BMP assay employed a mesophilic anaerobic culture obtained from the Terminal Island Sewage Treatment Plant, Terminal Island, CA. This anaerobic culture was assayed prior to use as detailed in Table 3.

Table 3. Analysis of the Terminal Island Anaerobic Culture Used in BMP Assays

Assay	Value
Total Solids	3.11%
Volatile Solids	62.53%
Ash	37.47%
pH	7.43

The BMP assays were prepared in triplicate using serum bottles with a total volume of 162 mL. Using a 25 mL pipette, 100 mL (\pm 1.8 mL) of active anaerobic culture was transferred to each serum bottle. The headspace of each serum bottle was then flushed with UHP nitrogen for 1-min. prior to closing the bottles with a rubber stopper and an aluminum crimp cap. The serum bottles were incubated at 37°C with shaking (200 rpm) using a Lab-Line Orbit Environ-Shaker.



The serum bottles were incubated for a period of almost 4 days prior to commencing the BMP assay in order to reduce background biogas production from intrinsic organic matter contained in the anaerobic sludge culture. In order to reduce the negative effects of dilution on the BMP anaerobic culture, a standard 5 mL addition of each test substrate was used. This represented roughly a 5% dilution of the anaerobic culture. The actual organic loadings and theoretical methane potential for each substrate varied as per its relative composition as described below in Table 4.

Table 4. BMP Organic Loadings

Sample	Volume Added	Organic Loading		Theoretical Methane Yield (mL)**
		gVS/bottle*	gCOD/bottle	
Bacto Peptone	5 mL	0.271	0.287	100.45
BF 772014	5 mL	0.118	0.141	49.35
MTX 7F	5 mL	0.197	0.174	60.90
TiO ₂	5 mL	0.202	0.279	97.65
Control Hydrolyz.	5 mL	0.211	0.272	95.20

* Volatile solids loading corrected for sample dilution during pH adjustment.

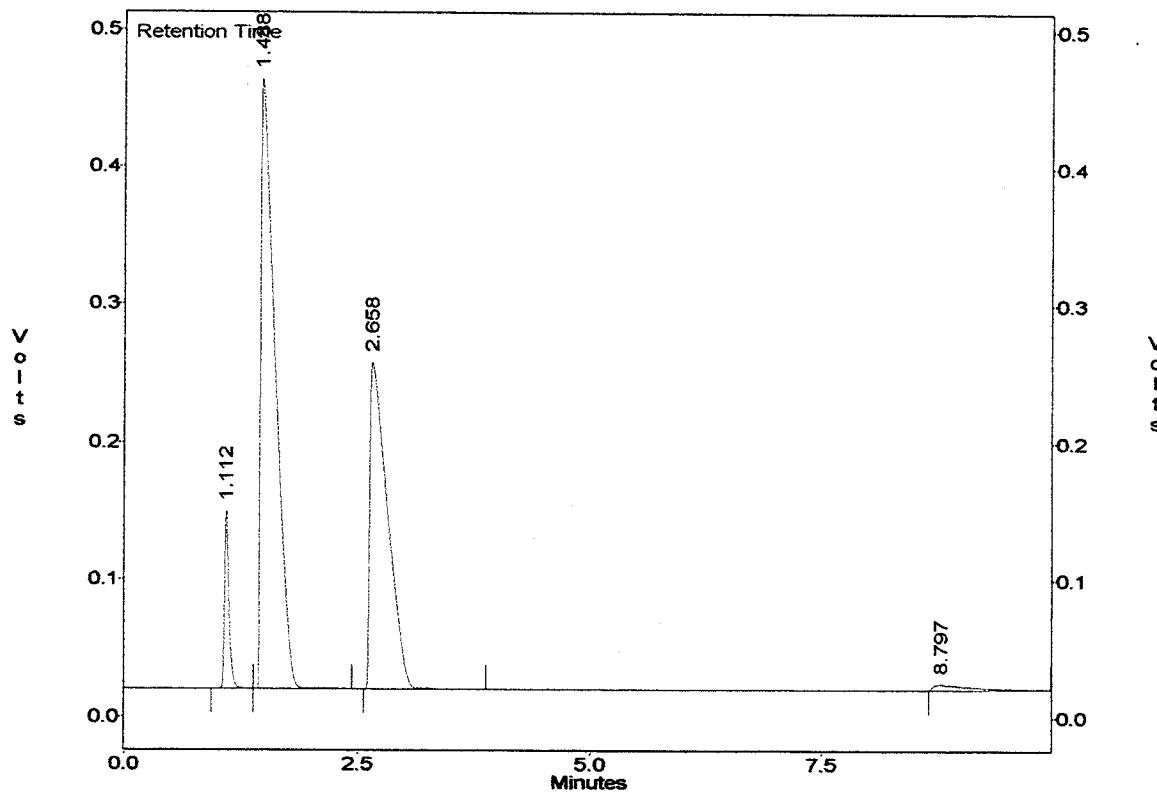
** Theoretical methane yields based on COD loading using a yield of 350 mL CH₄ per gram COD added (Owen and McCarty, 1964).

Biogas Analysis

Gas Analysis

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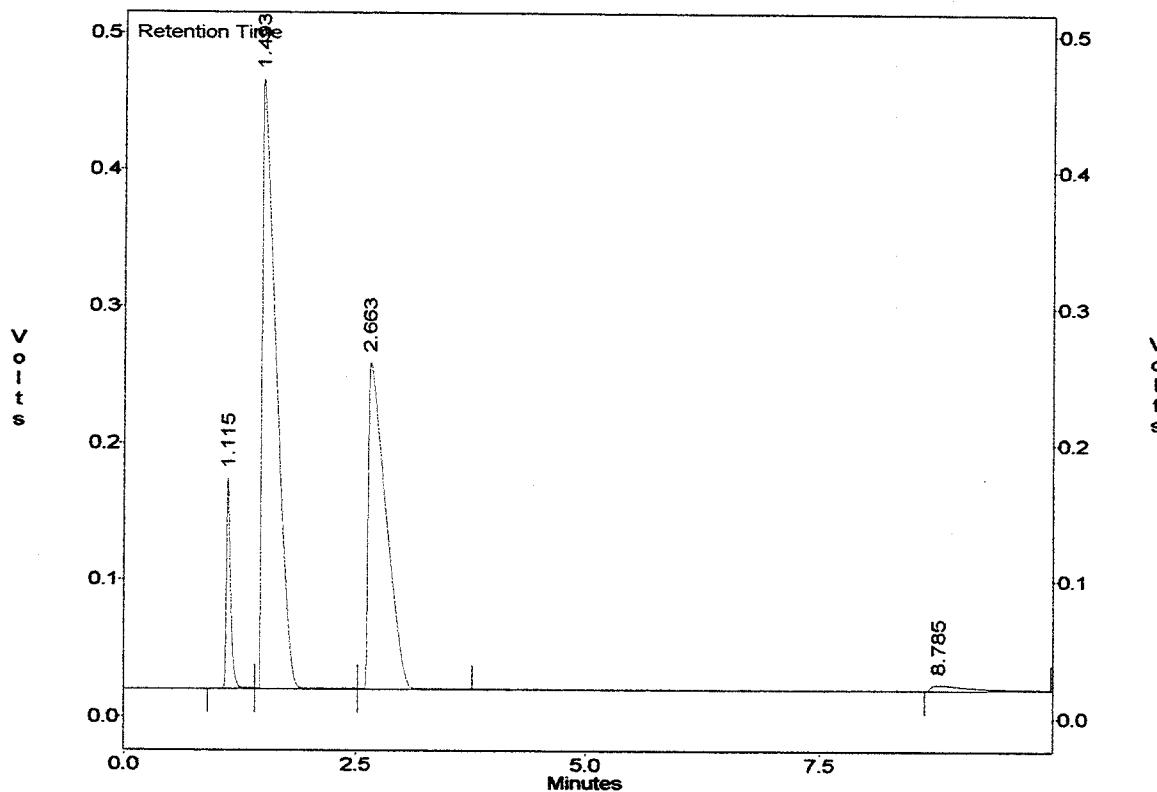
**Channel A Results**

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.488	4691432.5	67.69
3	Carbon Dioxide	2.658	3116158.0	32.31
Totals :			7807590.5	100.00

Gas Analysis

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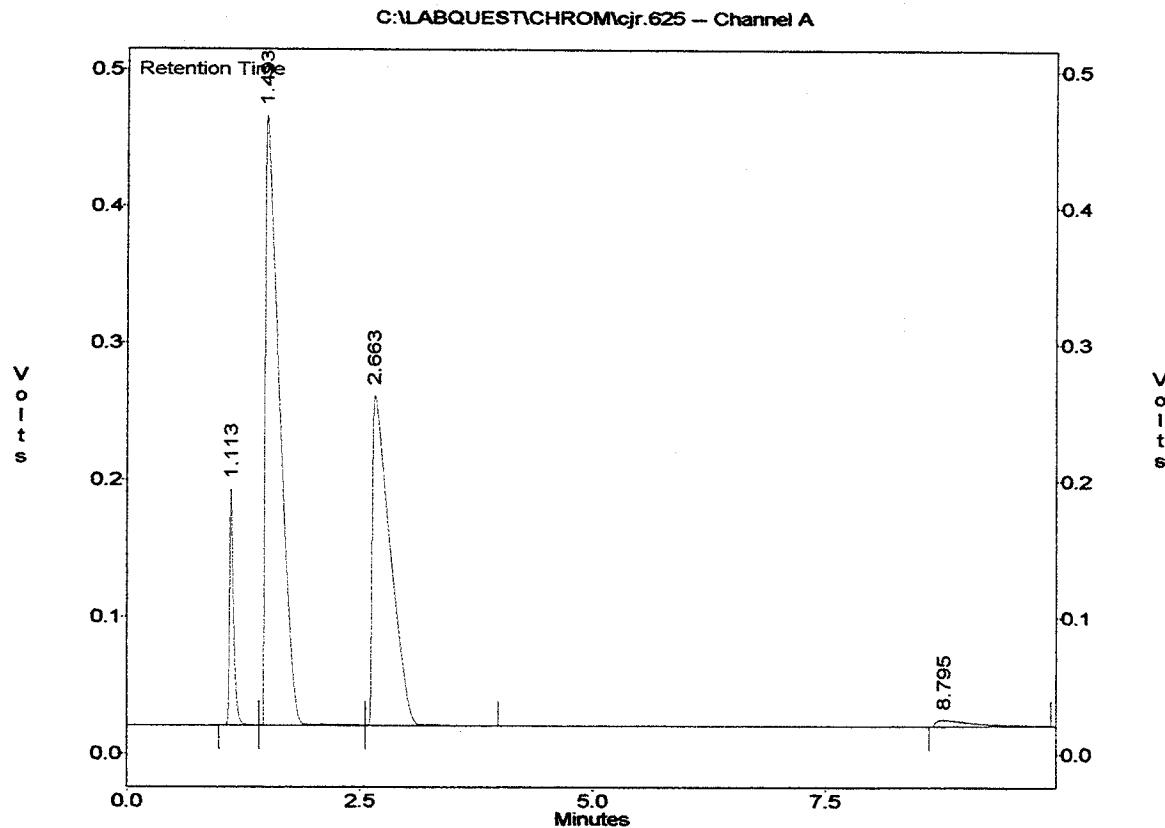
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**Channel A Results**

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.493	4724336.0	67.71
3	Carbon Dioxide	2.663	3135141.0	32.29
Totals :			7859477.0	100.00

Gas Analysis

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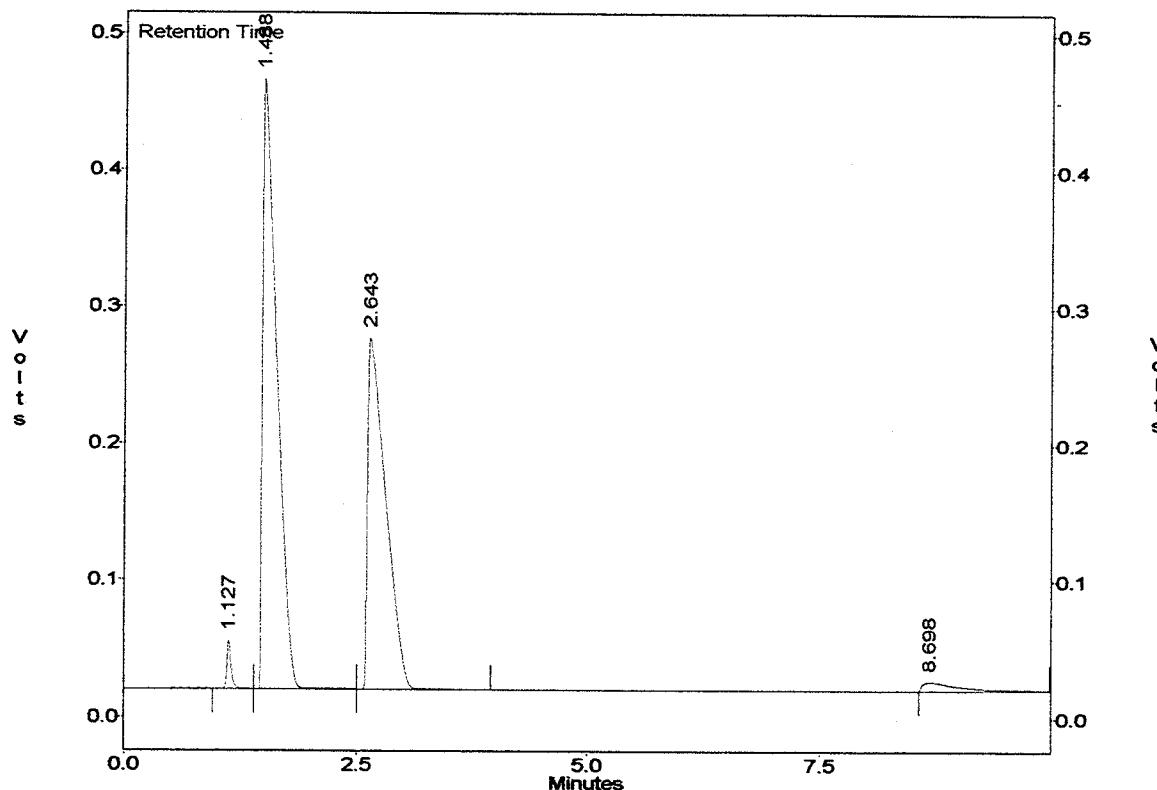
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.493	4714953.0	67.39
3	Carbon Dioxide	2.663	3175722.0	32.61
Totals :			7890675.0	100.00

Gas Analysis

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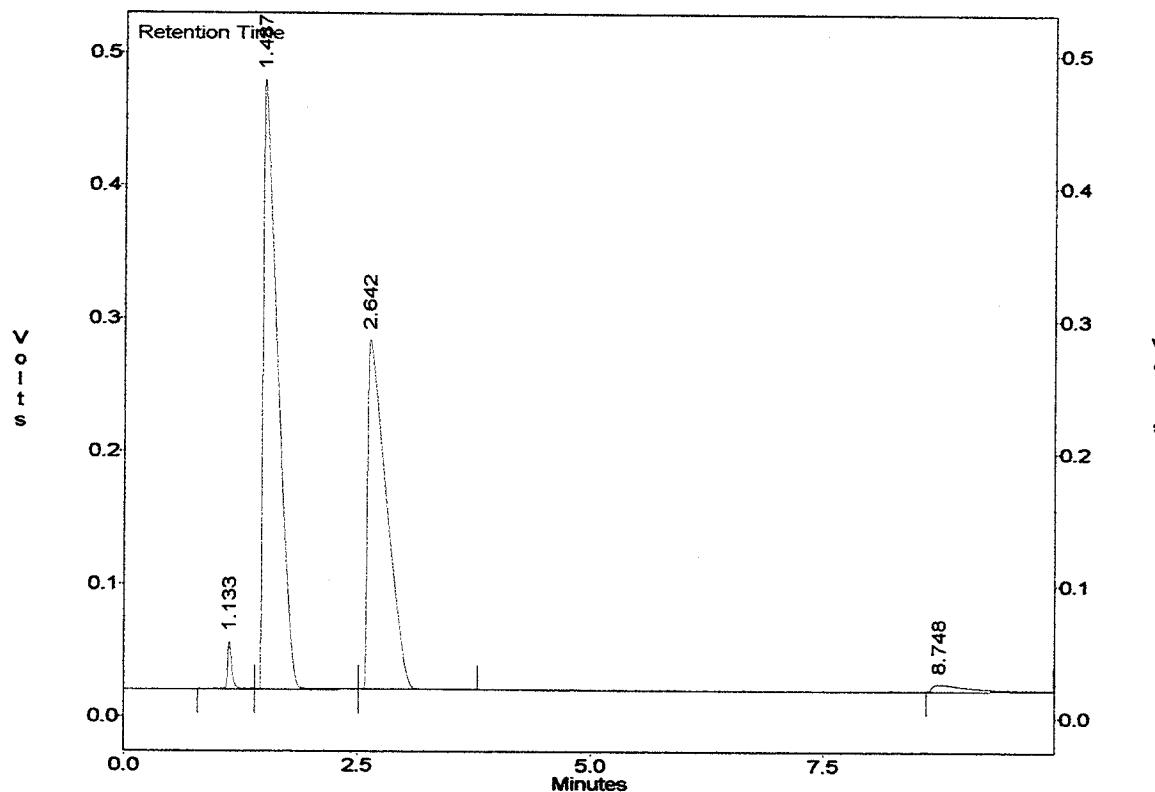
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.488	4693482.5	65.00
3	Carbon Dioxide	2.643	3517557.0	35.00
Totals :			8211039.5	100.00

Gas Analysis

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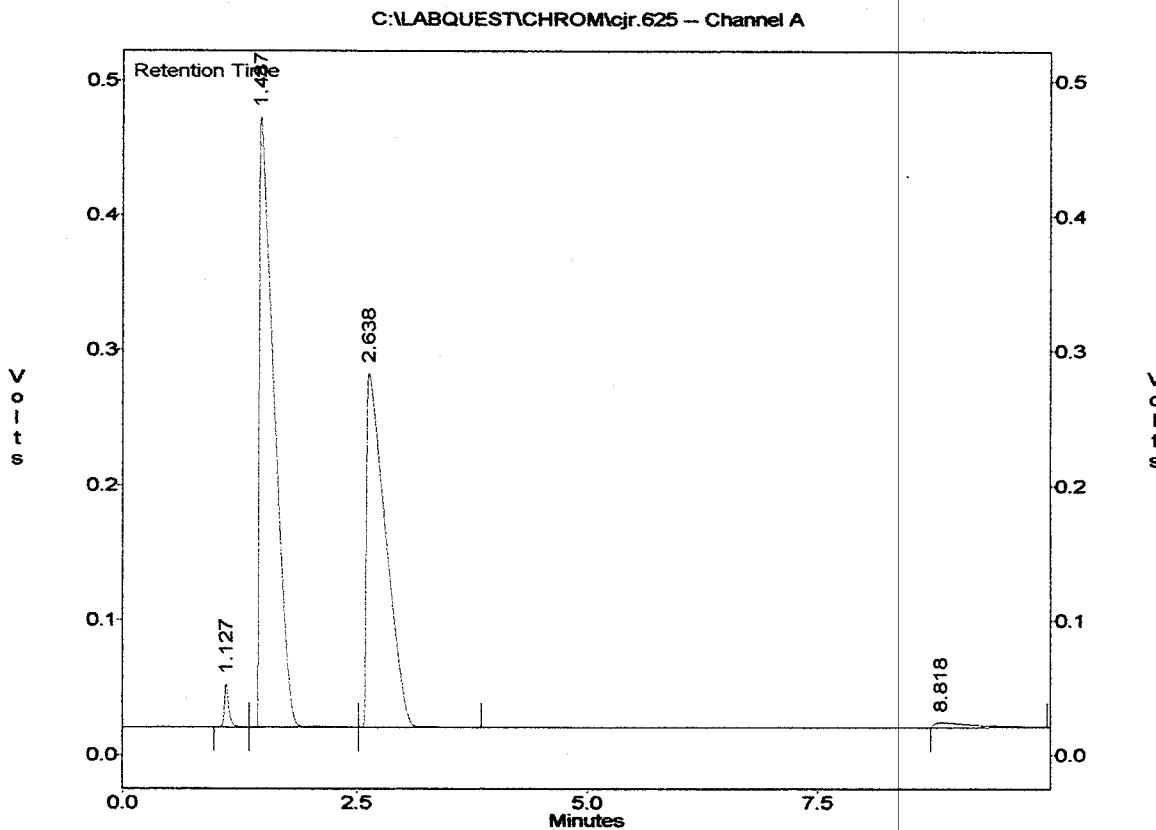


Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.487	4919626.5	65.17
3	Carbon Dioxide	2.642	3659567.8	34.83
Totals :			8579194.0	100.00

Gas Analysis

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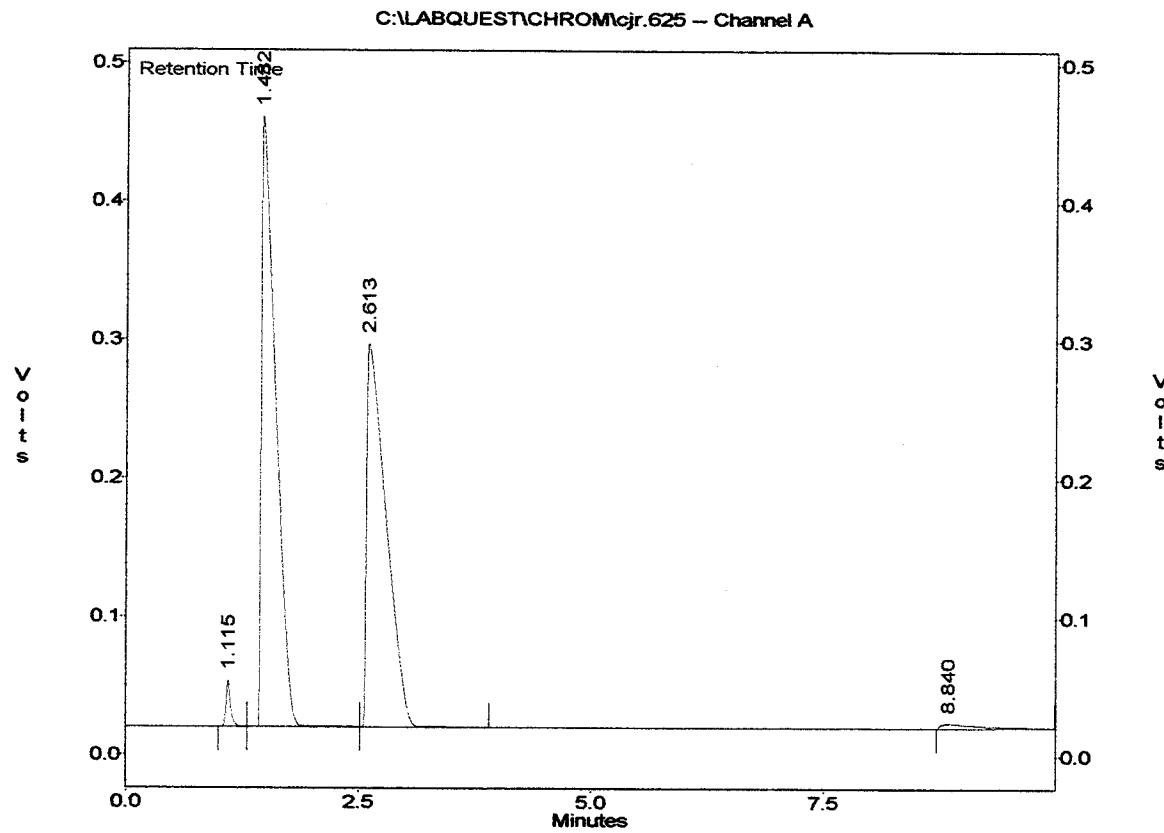


Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.487	4841500.0	64.78
3	Carbon Dioxide	2.638	3663040.0	35.22
Totals :			8504540.0	100.00

Gas Analysis

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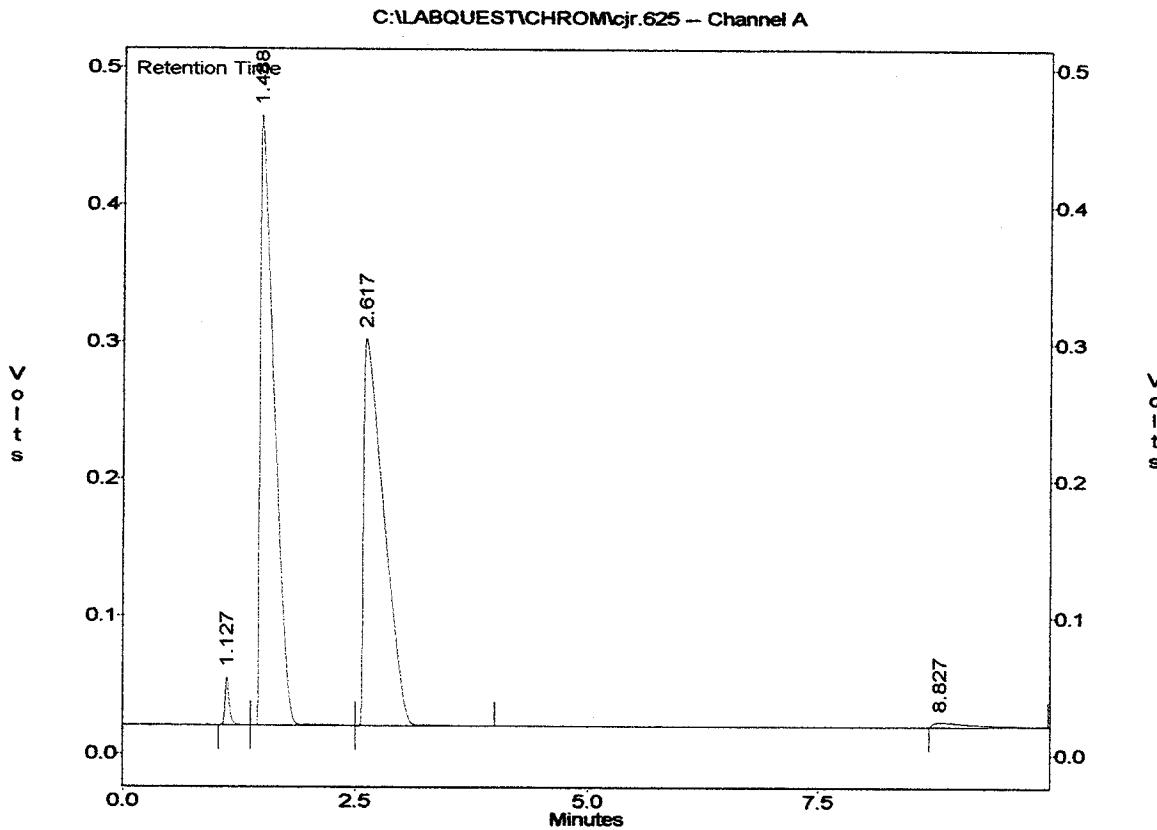


Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.482	4612954.0	61.70
3	Carbon Dioxide	2.613	3985246.5	38.30
Totals :			8598200.0	100.00

Gas Analysis

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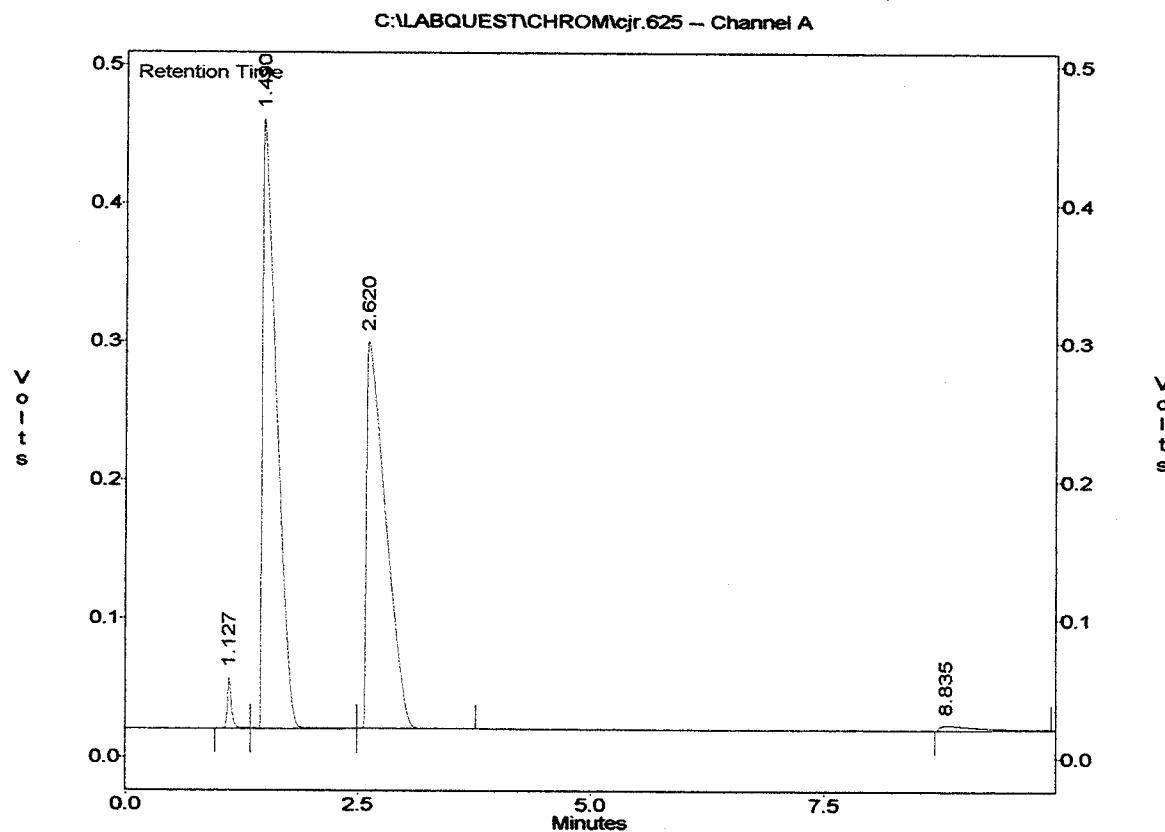


Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.488	4666704.0	61.28
3	Carbon Dioxide	2.617	4103121.5	38.72
Totals :			8769826.0	100.00

Gas Analysis

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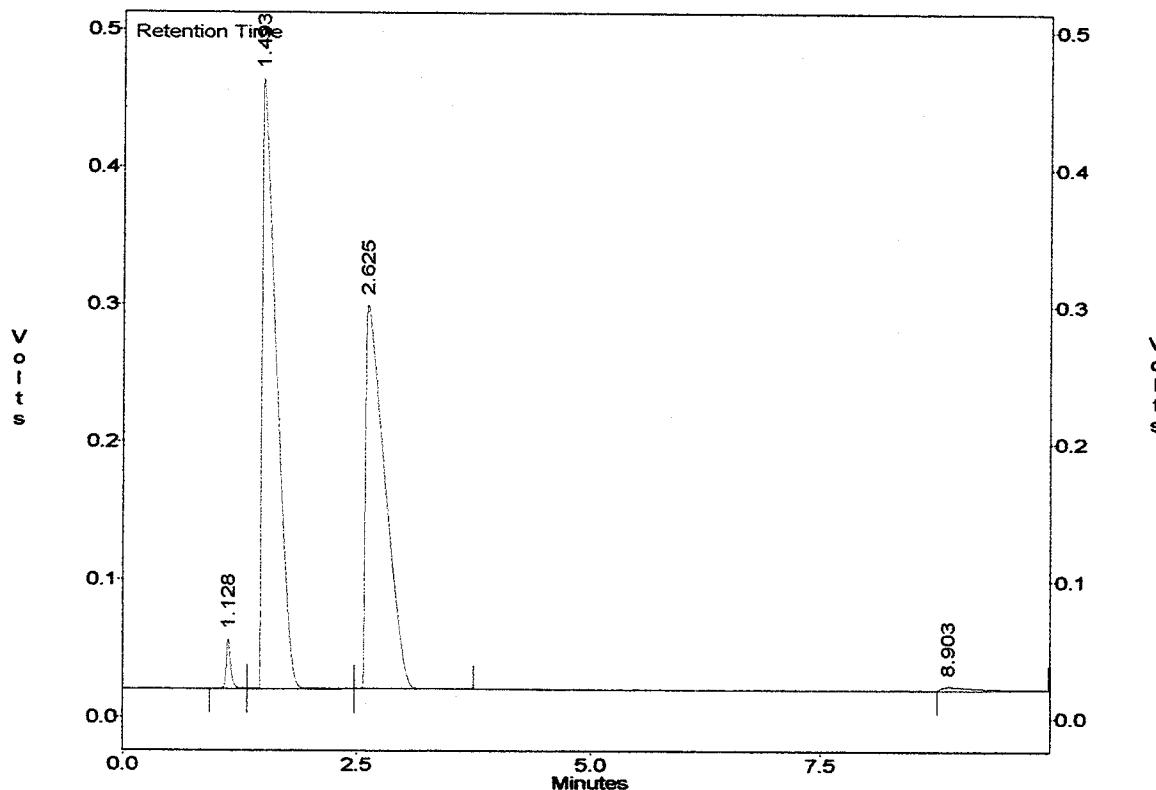
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.490	4582528.5	61.30
3	Carbon Dioxide	2.620	4026208.8	38.70
Totals :			8608737.0	100.00

Gas Analysis

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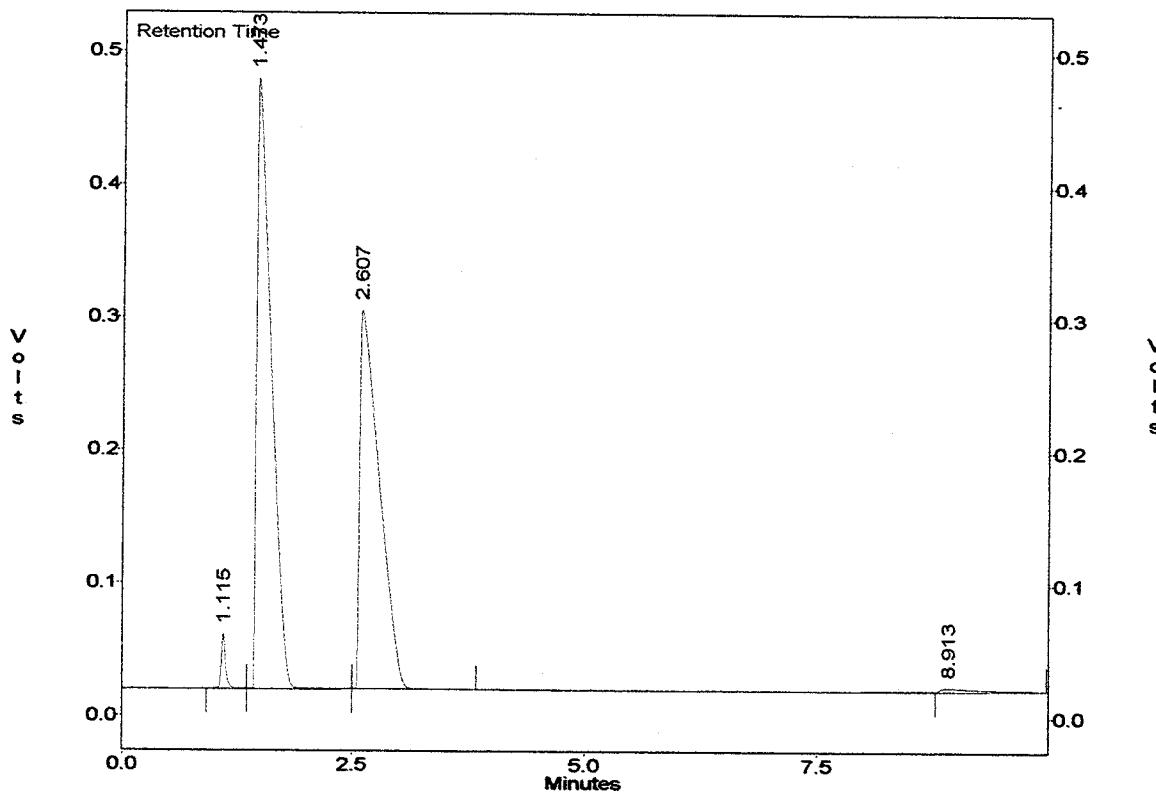
**Channel A Results**

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.493	4664040.5	61.66
3	Carbon Dioxide	2.625	4036362.8	38.34
Totals :			8700403.0	100.00

Gas Analysis

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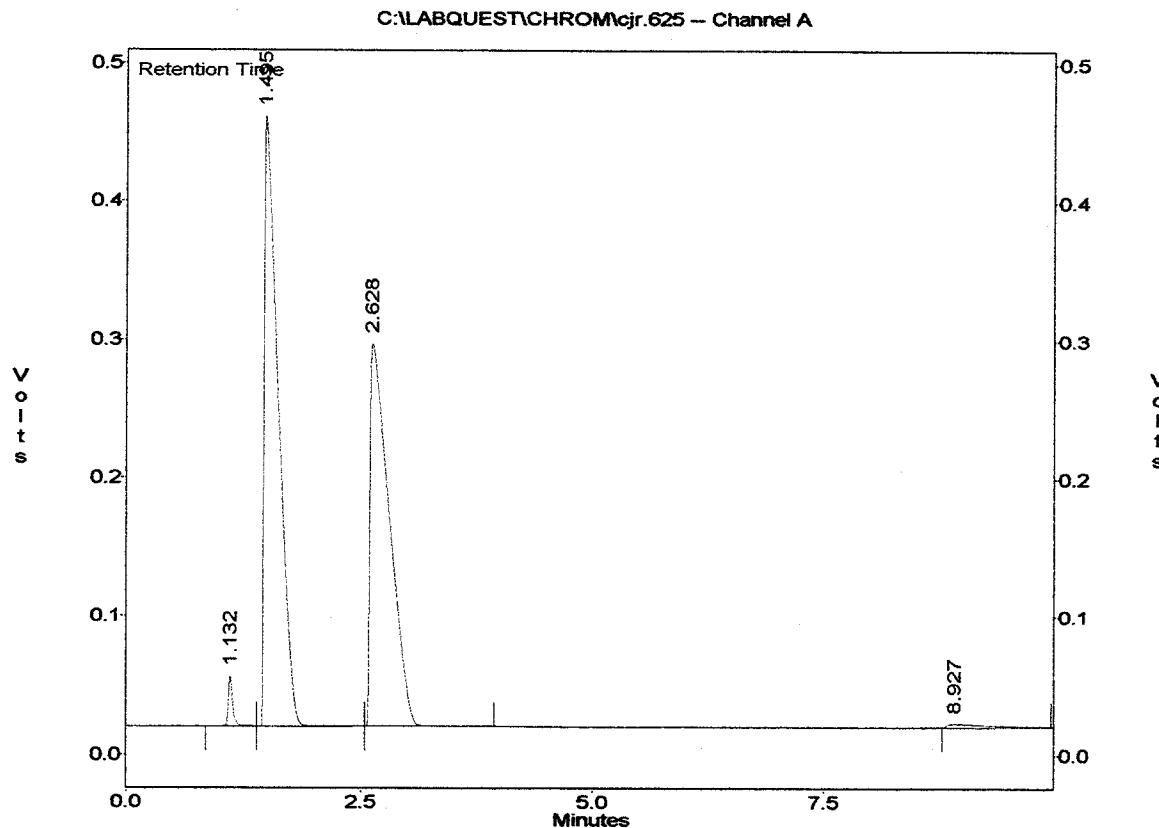


Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.473	4918913.0	62.21
3	Carbon Dioxide	2.607	4158397.5	37.79
Totals :			9077310.0	100.00

Gas Analysis

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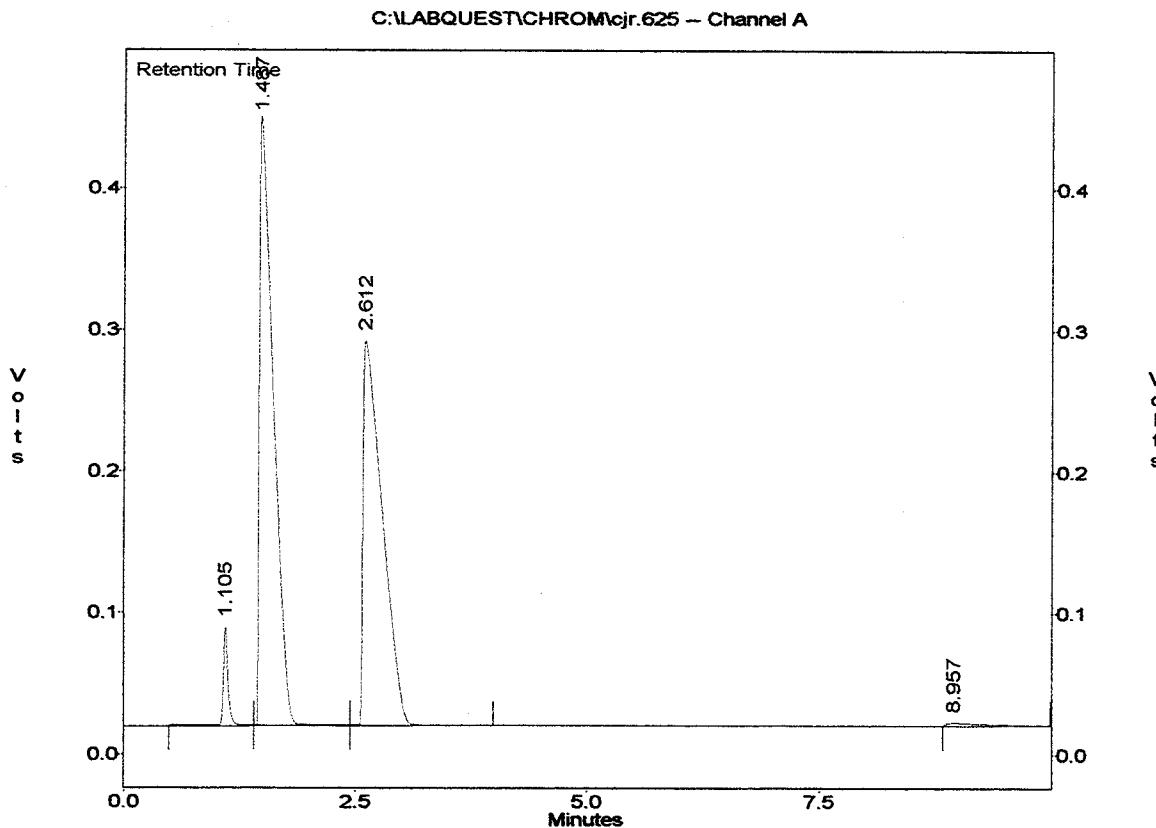


Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.495	4582253.0	61.72
3	Carbon Dioxide	2.628	3955167.8	38.28
Totals :			8537421.0	100.00

Gas Analysis

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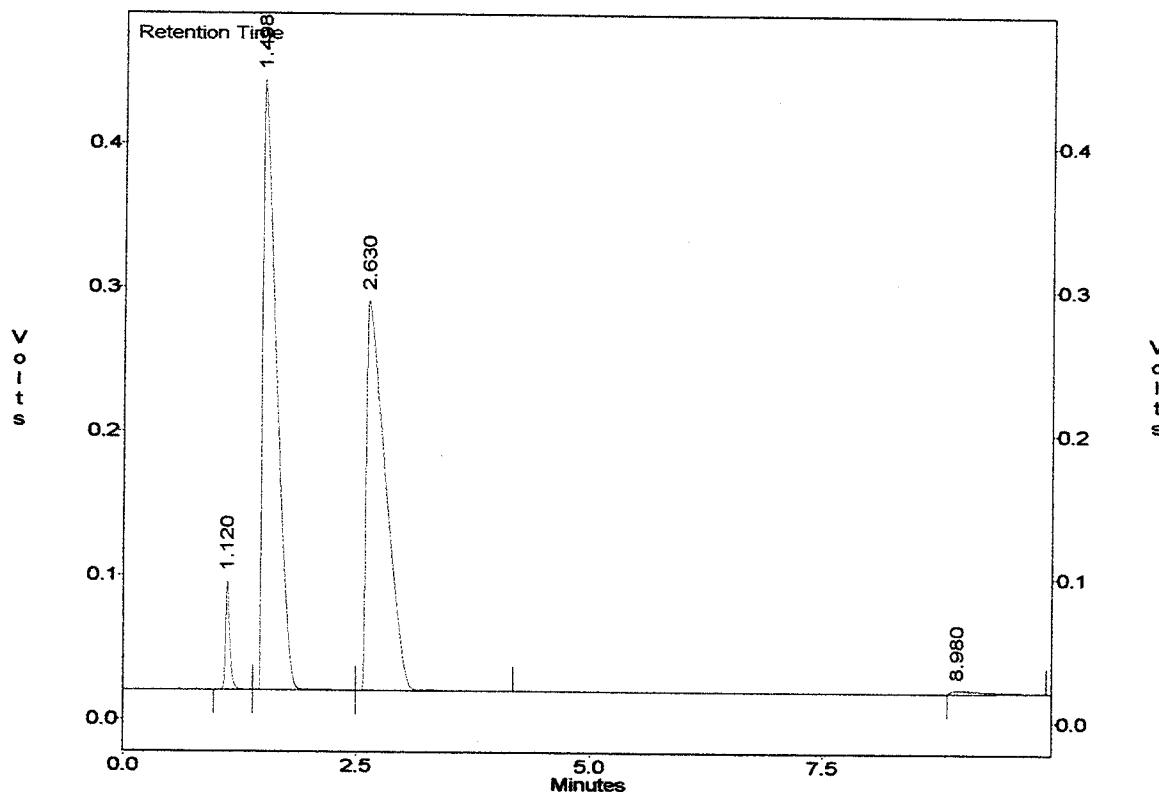
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.487	4431616.0	61.49
3	Carbon Dioxide	2.612	3862540.3	38.51
Totals :			8294156.0	100.00

Gas Analysis

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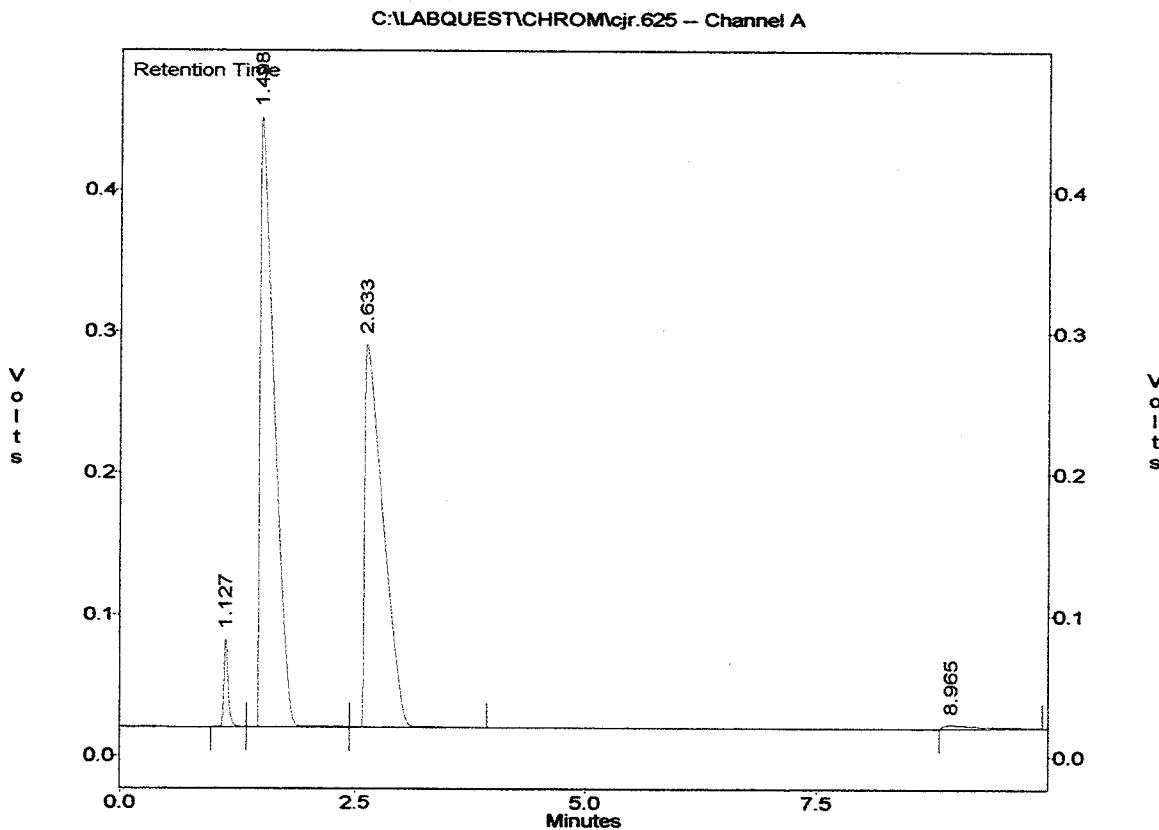


Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.498	4286963.0	61.03
3	Carbon Dioxide	2.630	3810111.0	38.97
Totals :			8097074.0	100.00

Gas Analysis

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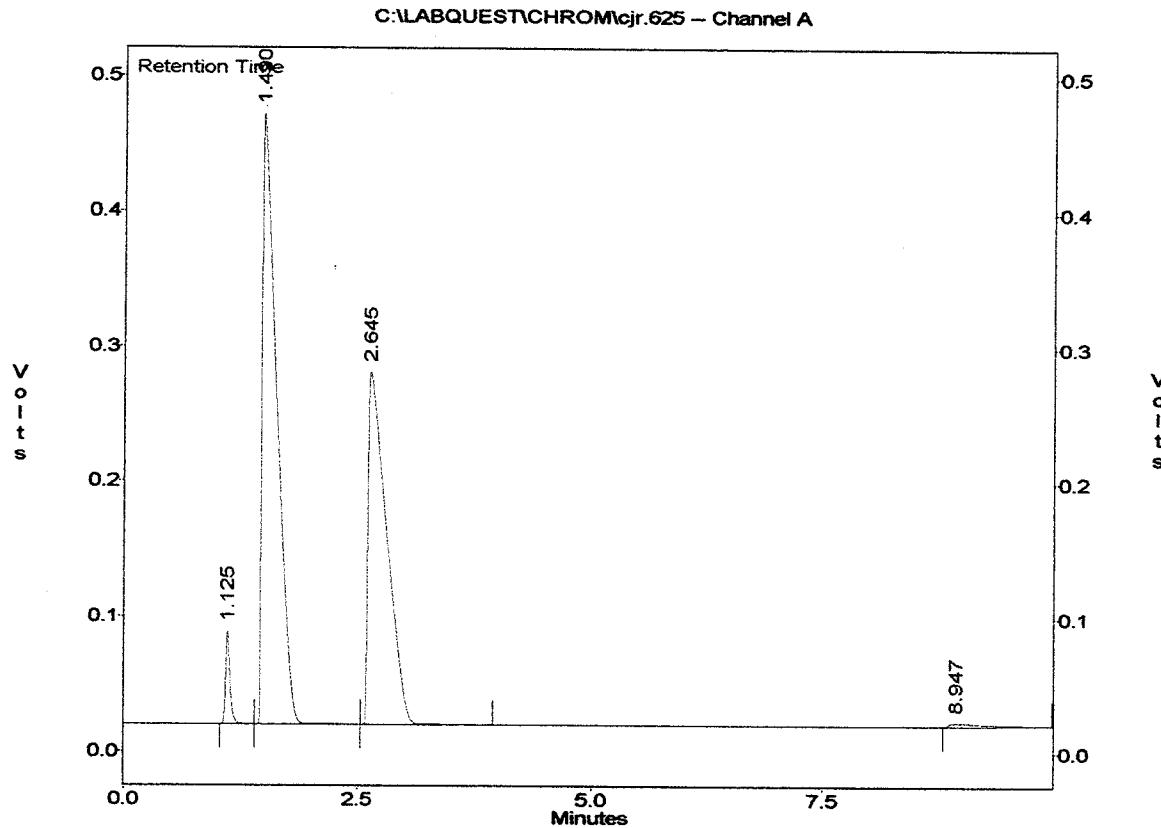


Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.498	4404101.5	61.68
3	Carbon Dioxide	2.633	3807966.8	38.32
Totals :				8212068.0 100.00

Gas Analysis

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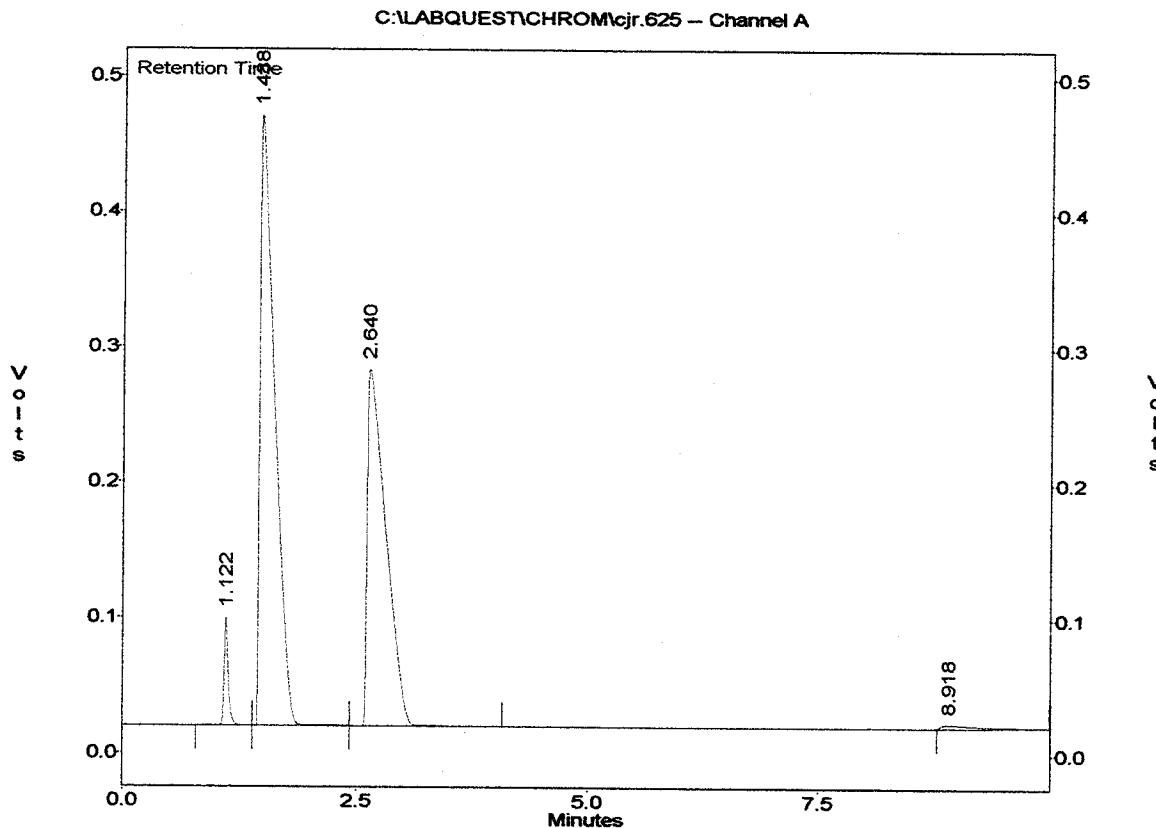


Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.490	4794559.5	64.89
3	Carbon Dioxide	2.645	3610497.5	35.11
Totals :			8405057.0	100.00

Gas Analysis

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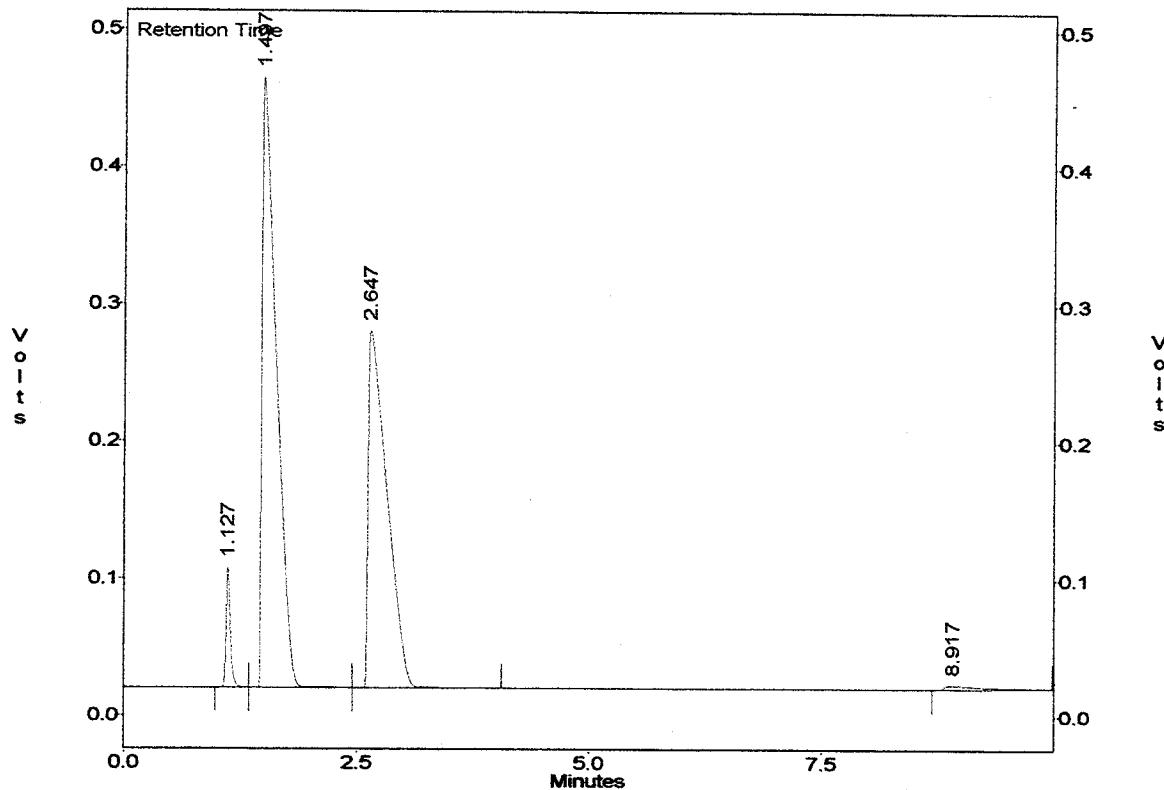
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.488	4767781.5	64.45
3	Carbon Dioxide	2.640	3659716.5	35.55
Totals :			8427498.0	100.00

Gas Analysis

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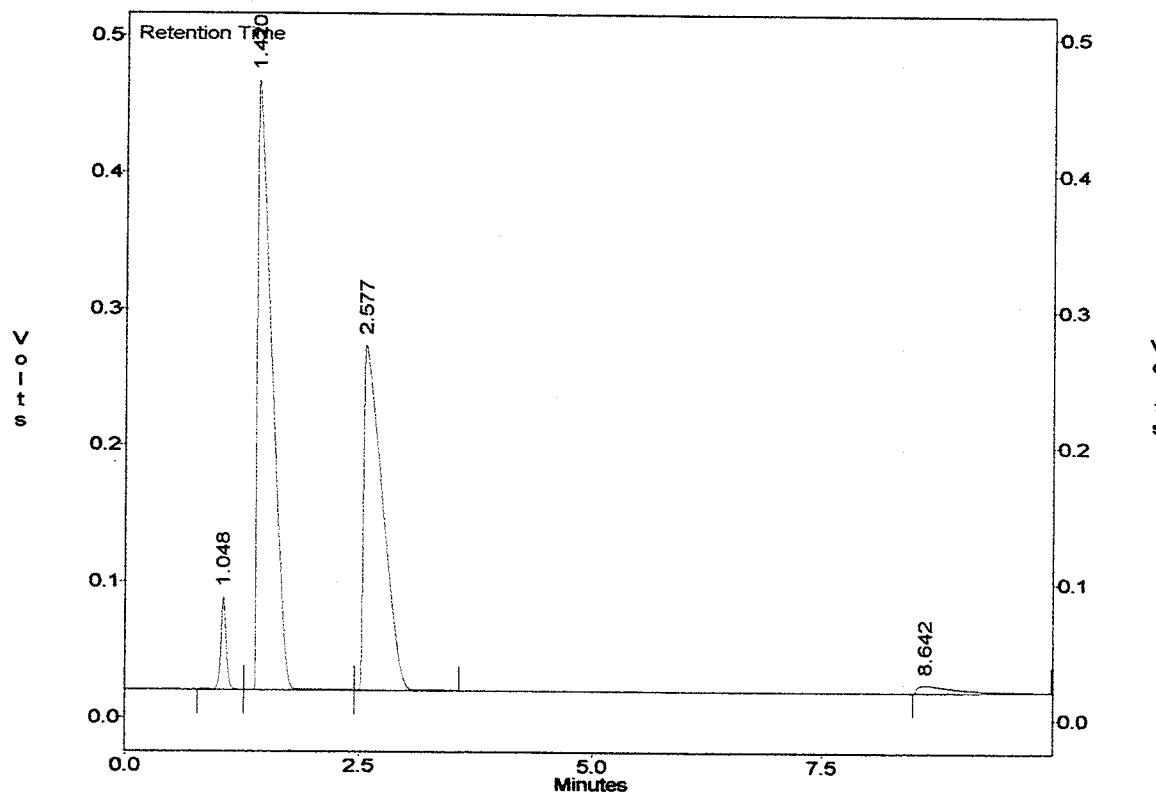
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.497	4672798.0	64.33
3	Carbon Dioxide	2.647	3605458.5	35.67
Totals :			8278256.5	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.621
Method : C:\LABQUEST\METHODS\biogas.MET
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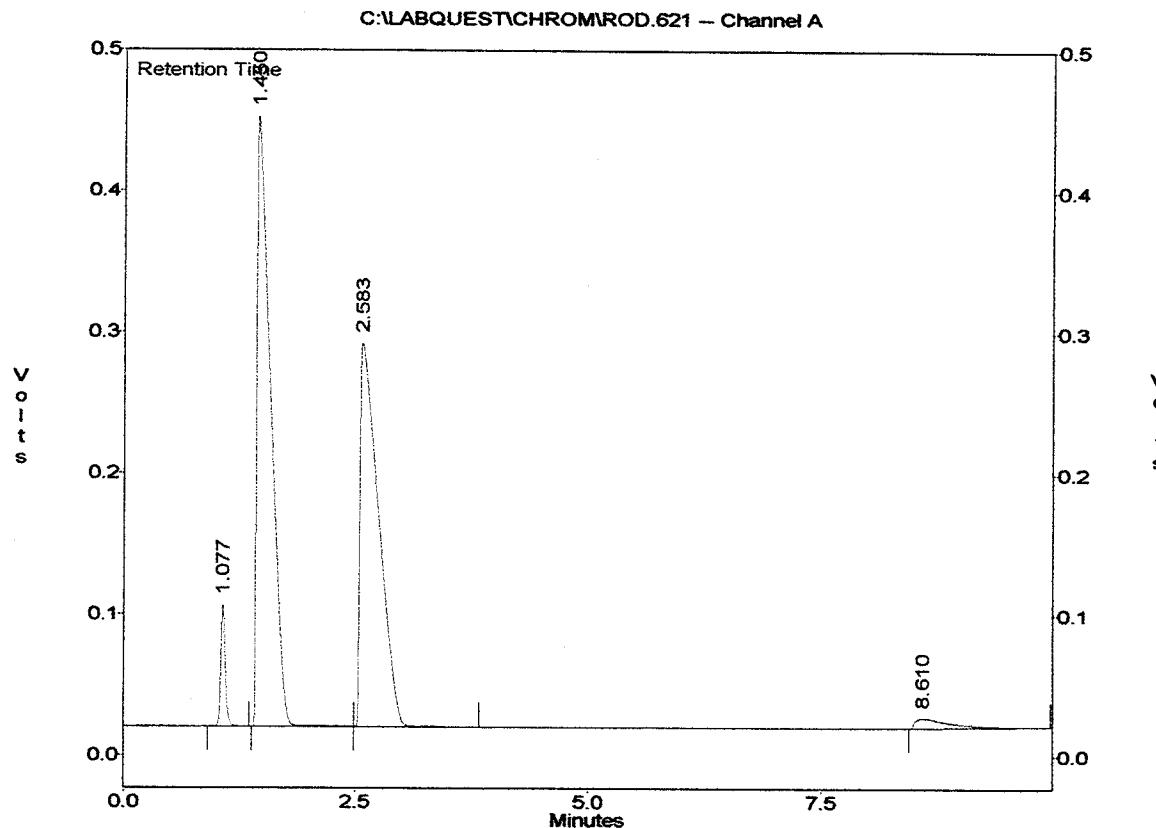


Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.420	4664980.0	65.53
3	Carbon Dioxide	2.577	3415075.5	34.47
Totals :			8080055.5	100.00

Gas Analysis

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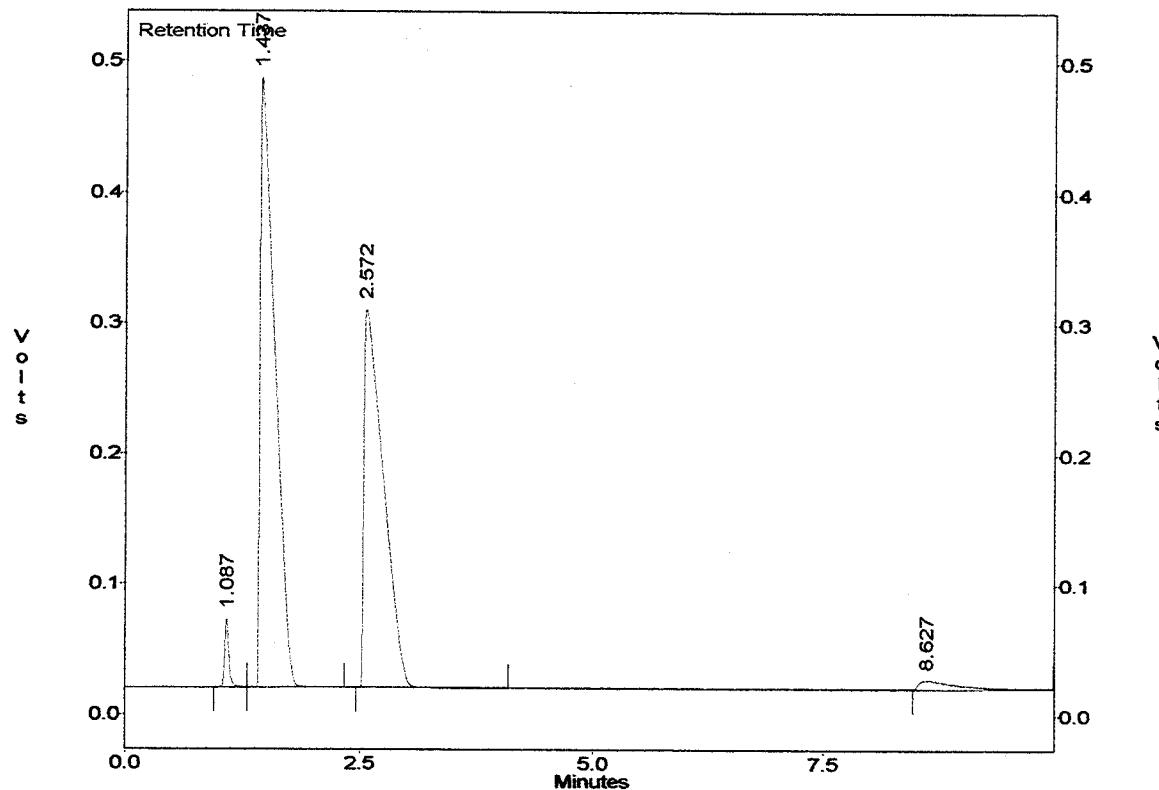
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.450	4351107.0	61.66
3	Carbon Dioxide	2.583	3764813.3	38.34
Totals :			8115920.0	100.00

Gas Analysis

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Method : C:\LABQUEST\METHODS\biogas.MET
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Printed : Jun 22, 1998 00:22:22

C:\LABQUEST\CHROM\ROD.621 -- Channel A



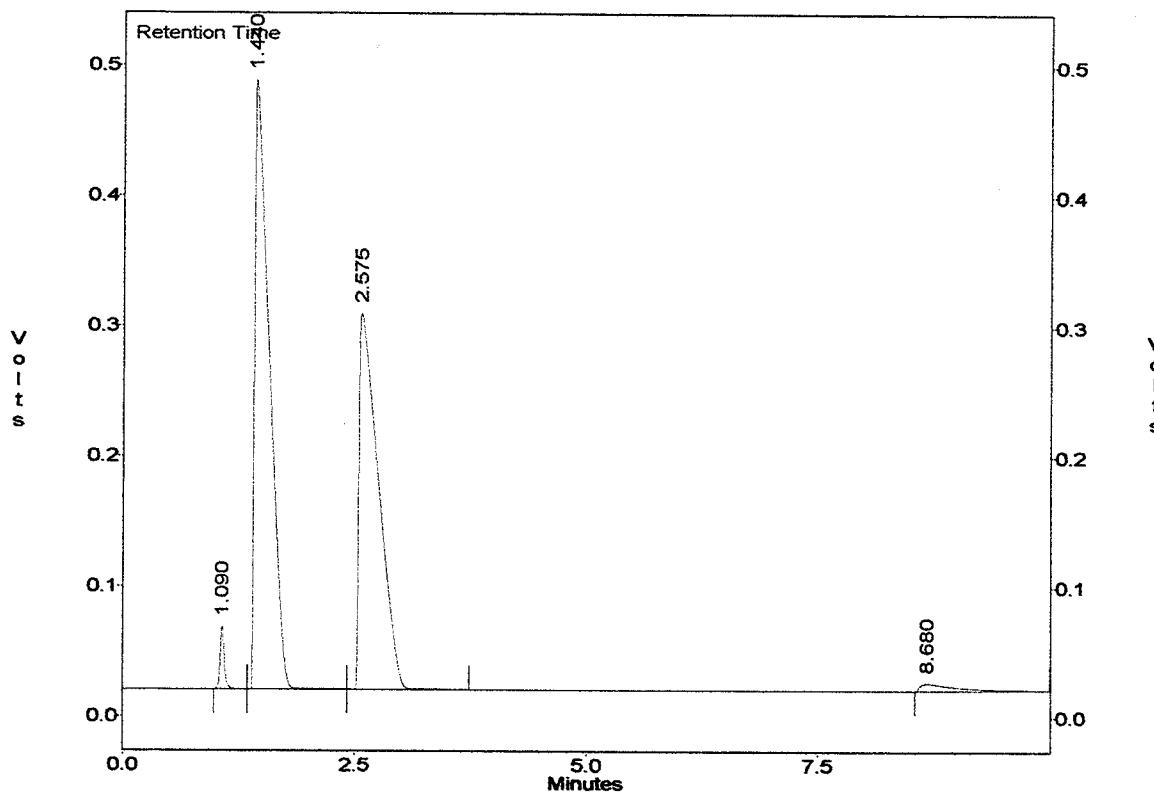
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.437	4935281.5	62.19
3	Carbon Dioxide	2.572	4175114.3	37.81
Totals :			9110396.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.621
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NERL / 7
Printed : Jun 22, 1998 00:04:08

C:\LABQUEST\CHROM\ROD.621 – Channel A



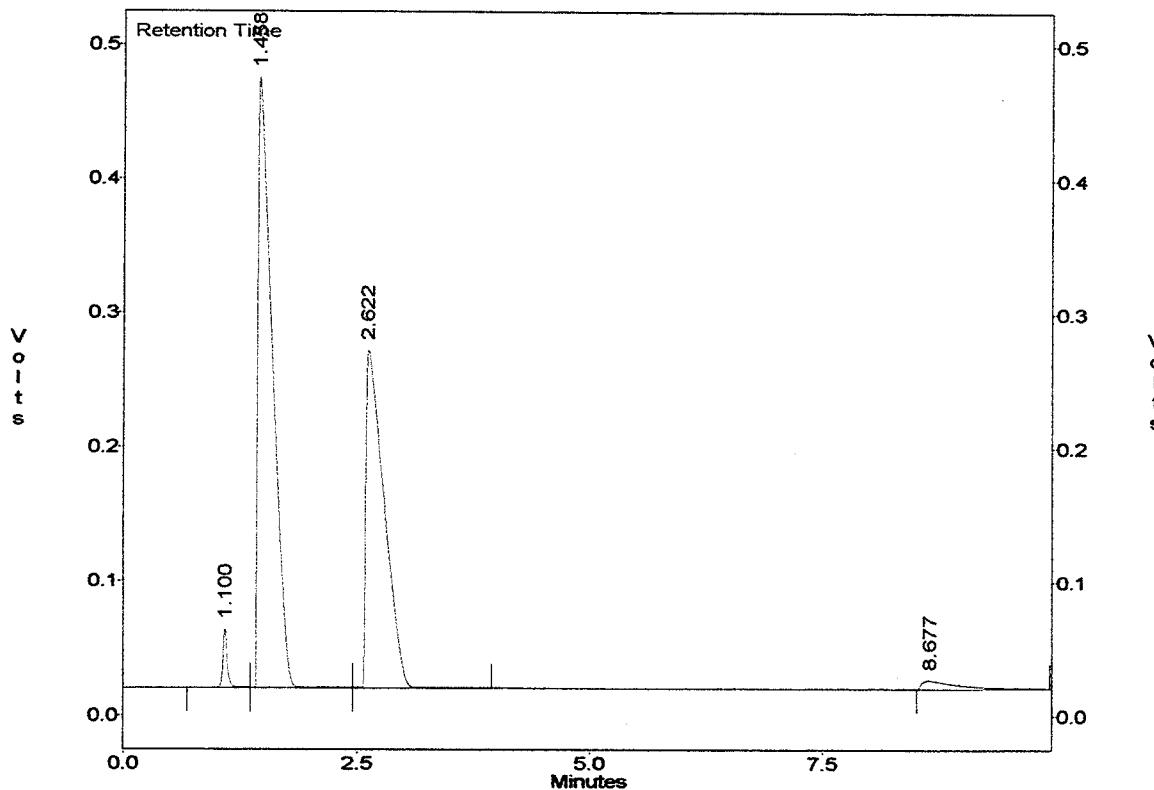
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.440	4952168.0	62.38
3	Carbon Dioxide	2.575	4156688.5	37.62
Totals :			9108856.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.621
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NERL / 4
Printed : Jun 21, 1998 23:37:12

C:\LABQUEST\CHROM\ROD.621 -- Channel A



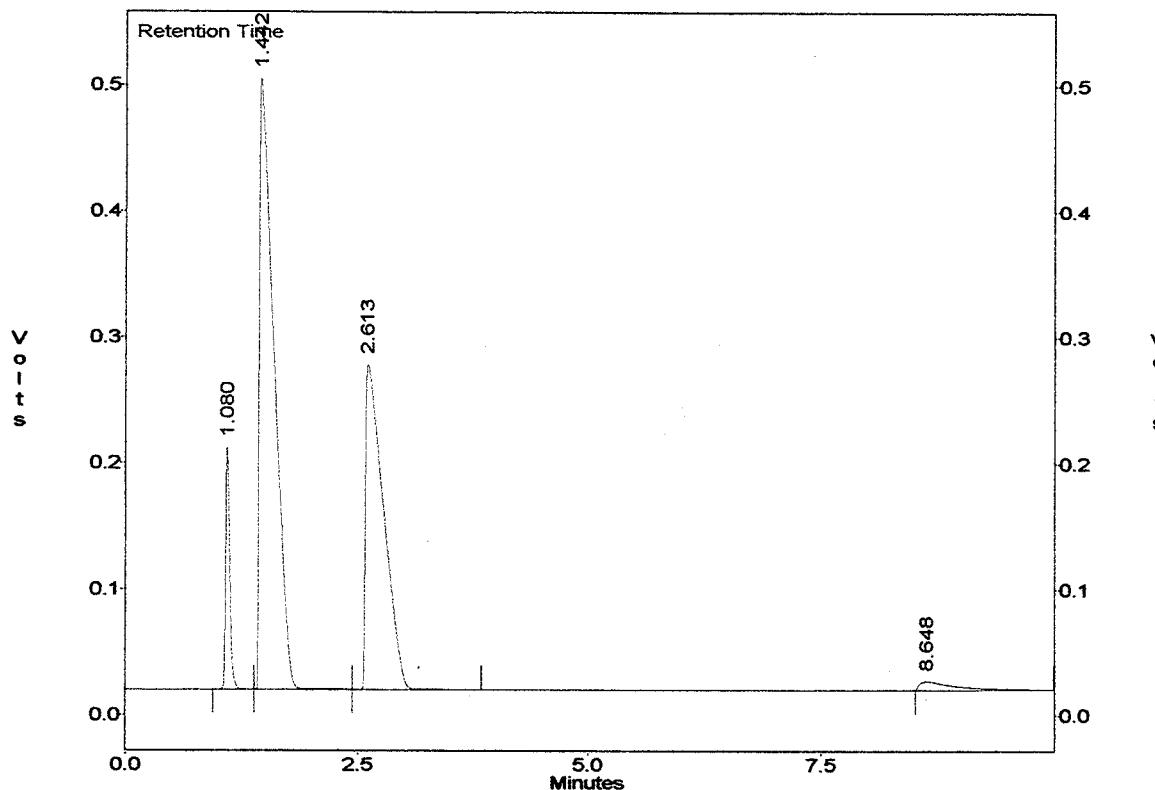
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.458	4751608.0	66.55
3	Carbon Dioxide	2.622	3323181.8	33.45
Totals :			8074790.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.621
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NERL / 1
Printed : Jun 21, 1998 23:09:03

C:\LABQUEST\CHROM\ROD.621 -- Channel A



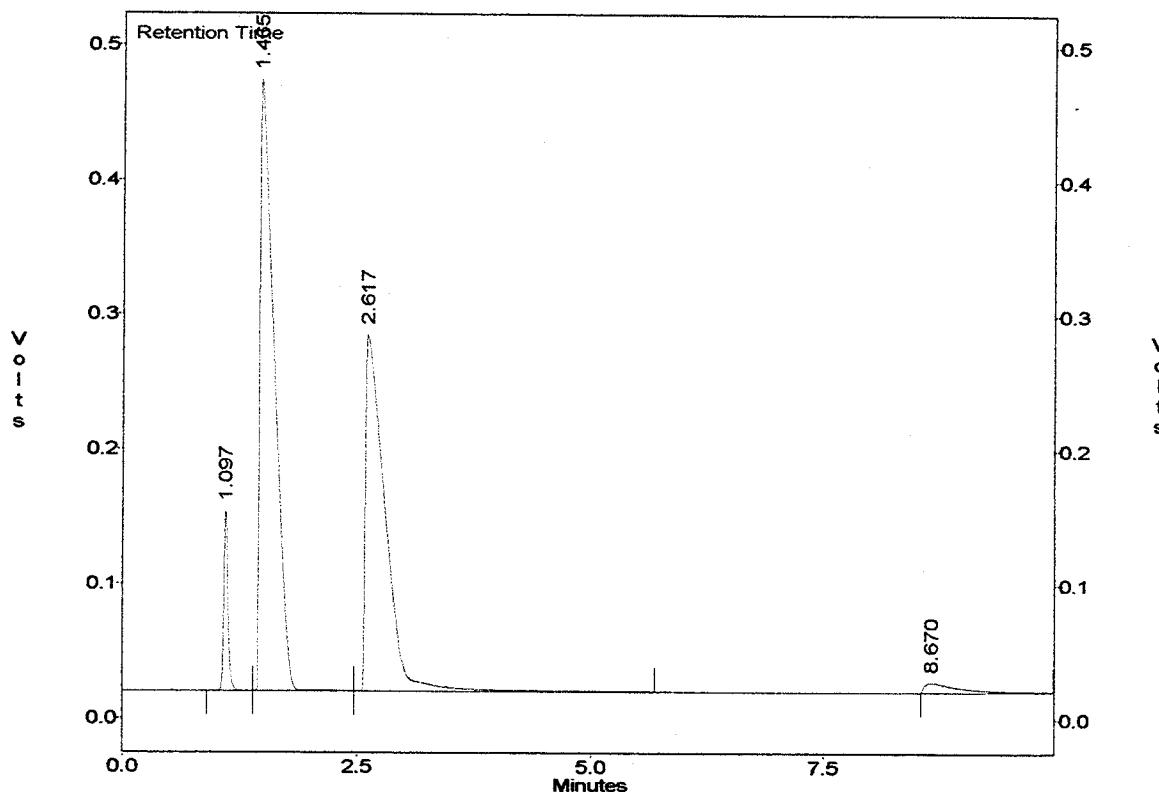
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.442	5291346.0	68.01
3	Carbon Dioxide	2.613	3463263.0	31.99
Totals :			8754609.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.613
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL / 16
Printed : Jun 13, 1998 22:36:09

C:\LABQUEST\CHROM\ROD.613 - Channel A



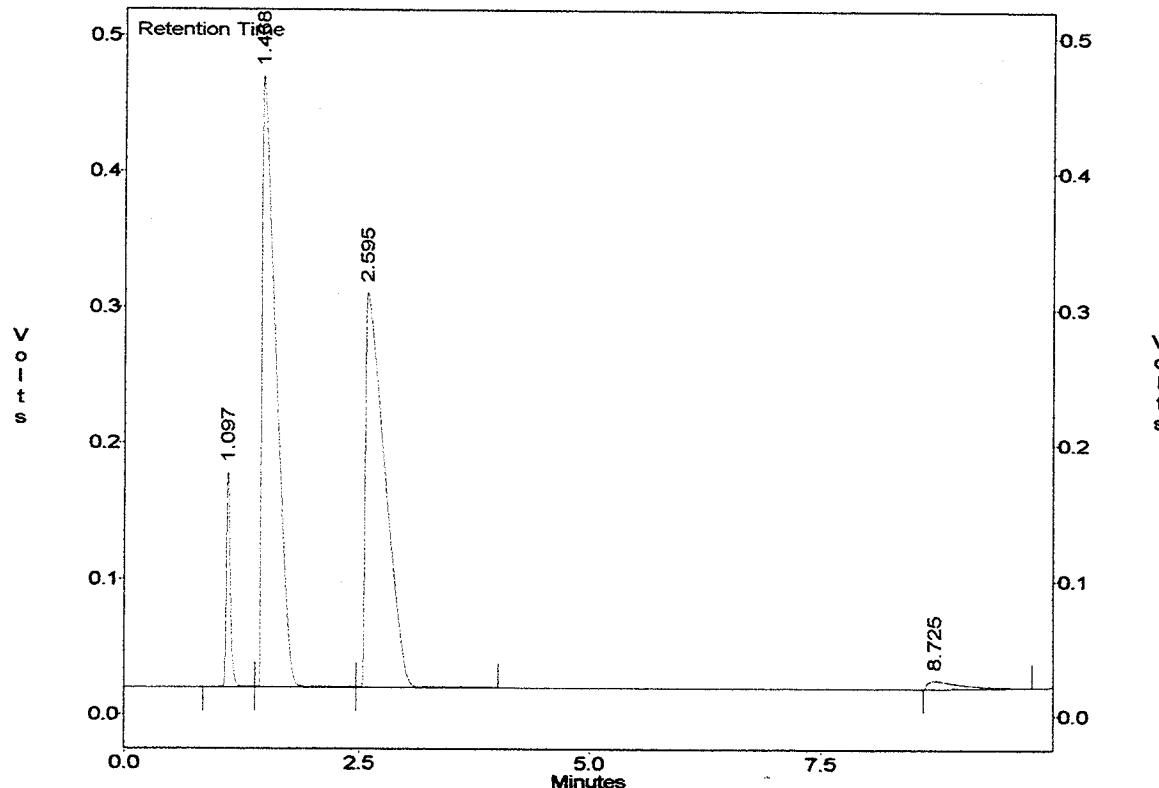
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.465	4714369.0	63.07
3	Carbon Dioxide	2.617	3841557.8	36.93
Totals :			8555927.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.613
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL / 13
Printed : Jun 13, 1998 22:06:28

C:\LABQUEST\CHROM\ROD.613 -- Channel A



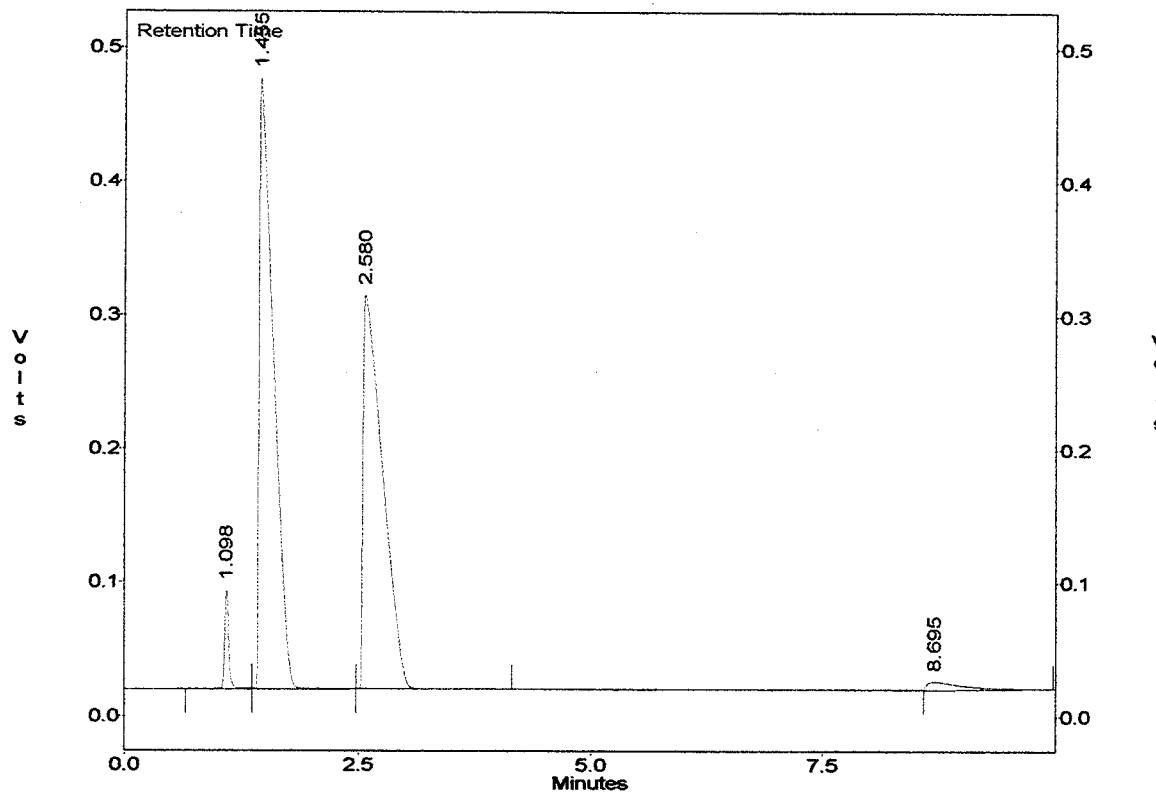
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.468	4625750.5	60.57
3	Carbon Dioxide	2.595	4191349.0	39.43
Totals :			8817100.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.613
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL / 10
Printed : Jun 13, 1998 21:55:19

C:\LABQUEST\CHROM\ROD.613 – Channel A



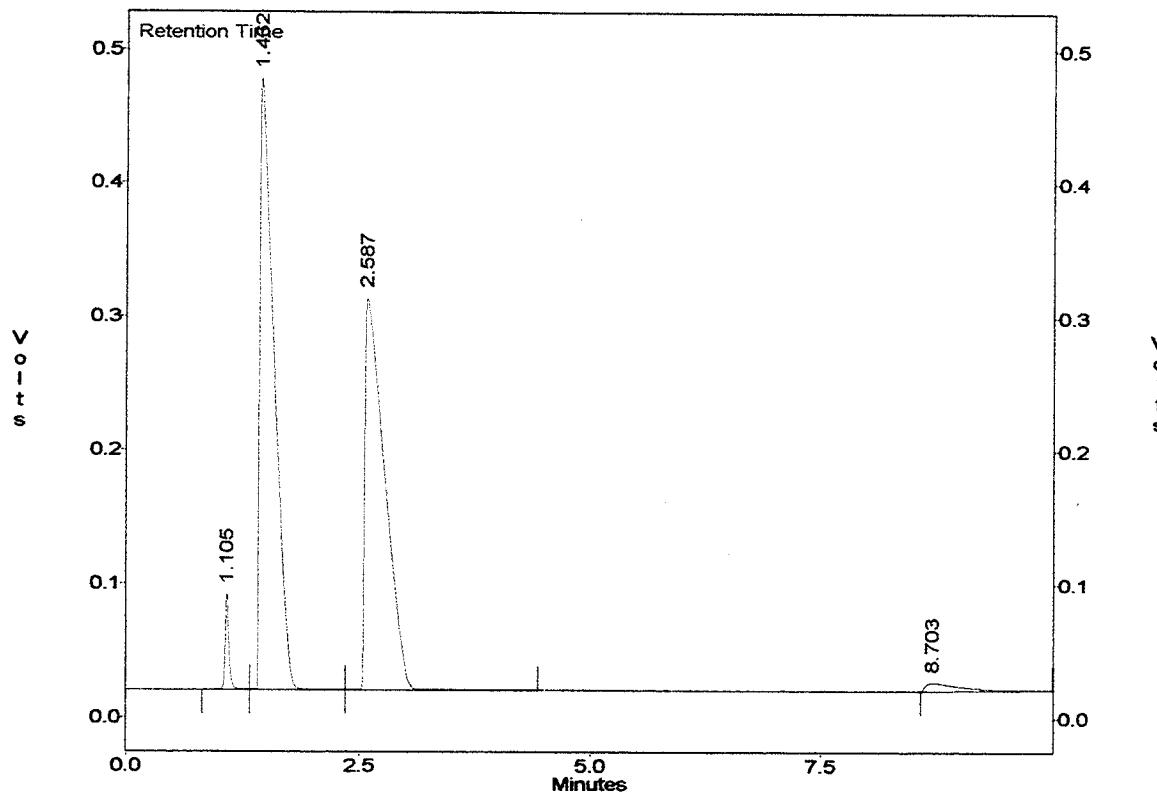
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.455	4755520.0	60.62
3	Carbon Dioxide	2.580	4299400.0	39.38
Totals :			9054920.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.613
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL / 7
Printed : Jun 13, 1998 21:14:13

C:\LABQUEST\CHROM\ROD.613 – Channel A



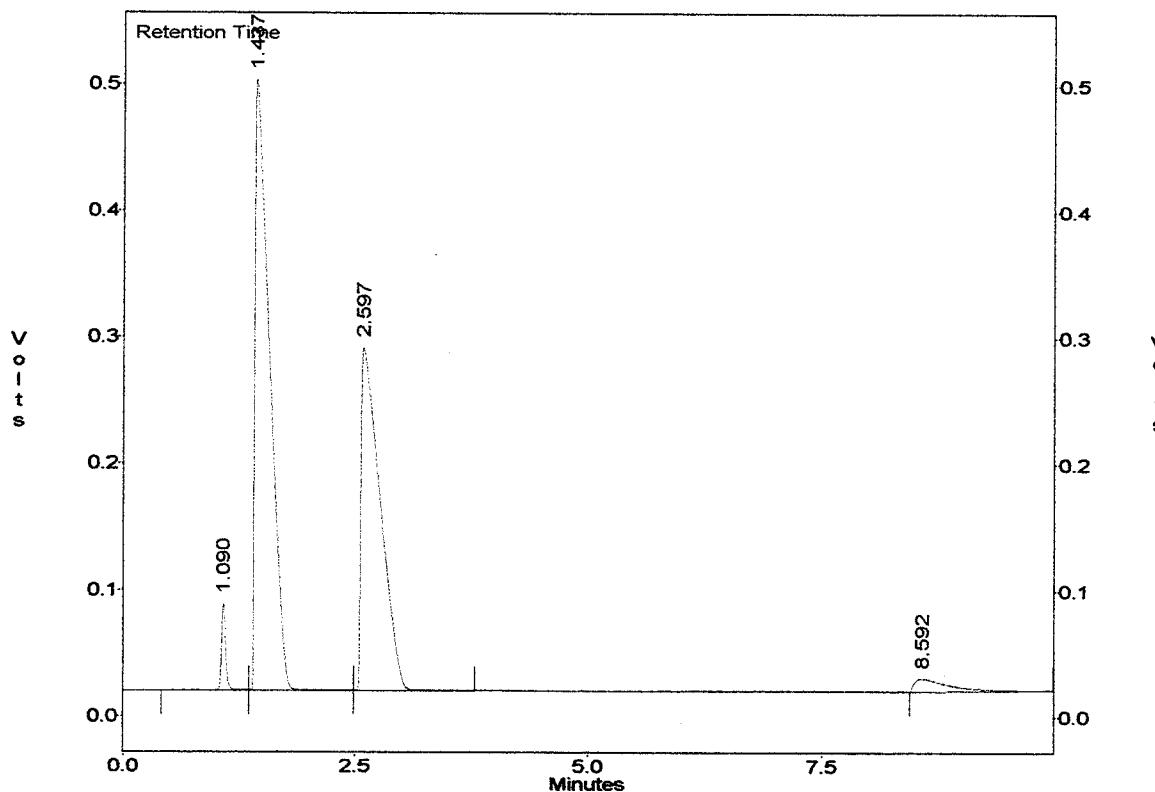
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.462	4768752.0	60.80
3	Carbon Dioxide	2.587	4278701.0	39.20
Totals :			9047453.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.613
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL / 4
Printed : Jun 13, 1998 20:17:59

C:\LABQUEST\CHROM\ROD.613 -- Channel A



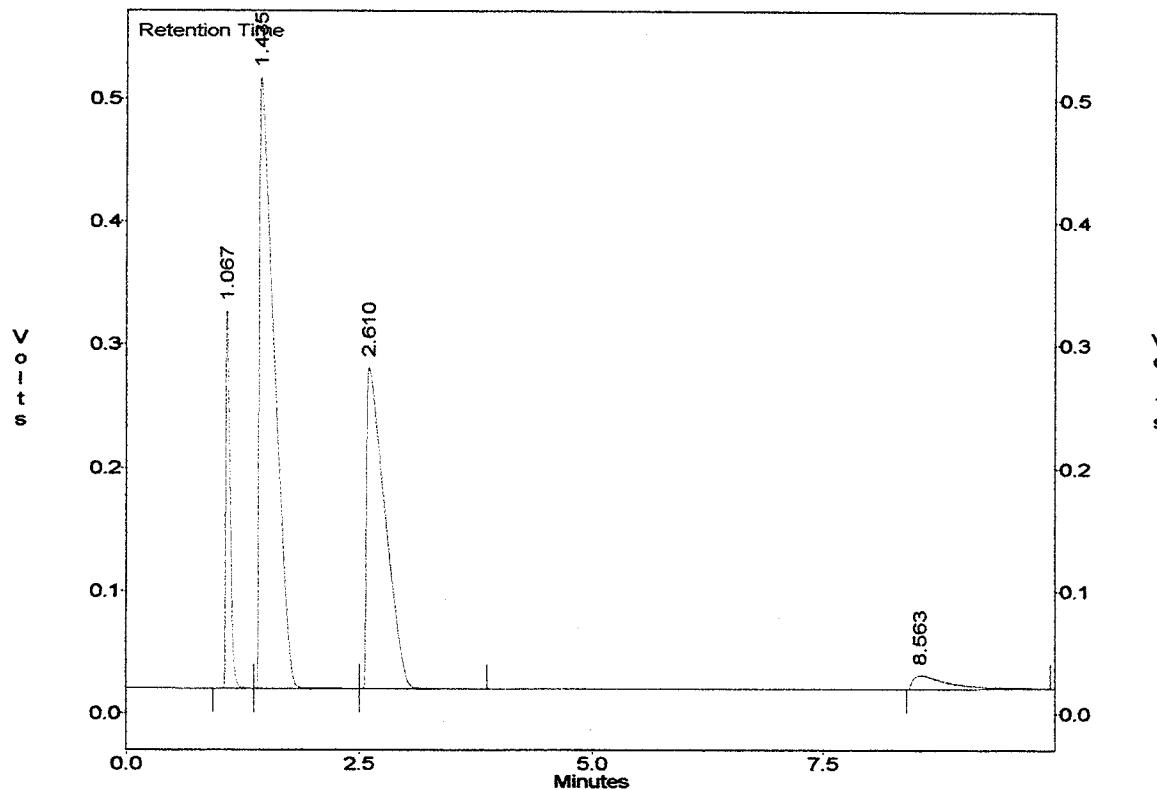
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.437	5242003.0	65.99
3	Carbon Dioxide	2.597	3759144.8	34.01
Totals :			9001148.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.613
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL / 1
Printed : Jun 13, 1998 19:41:23

C:\LABQUEST\CHROM\ROD.613 – Channel A



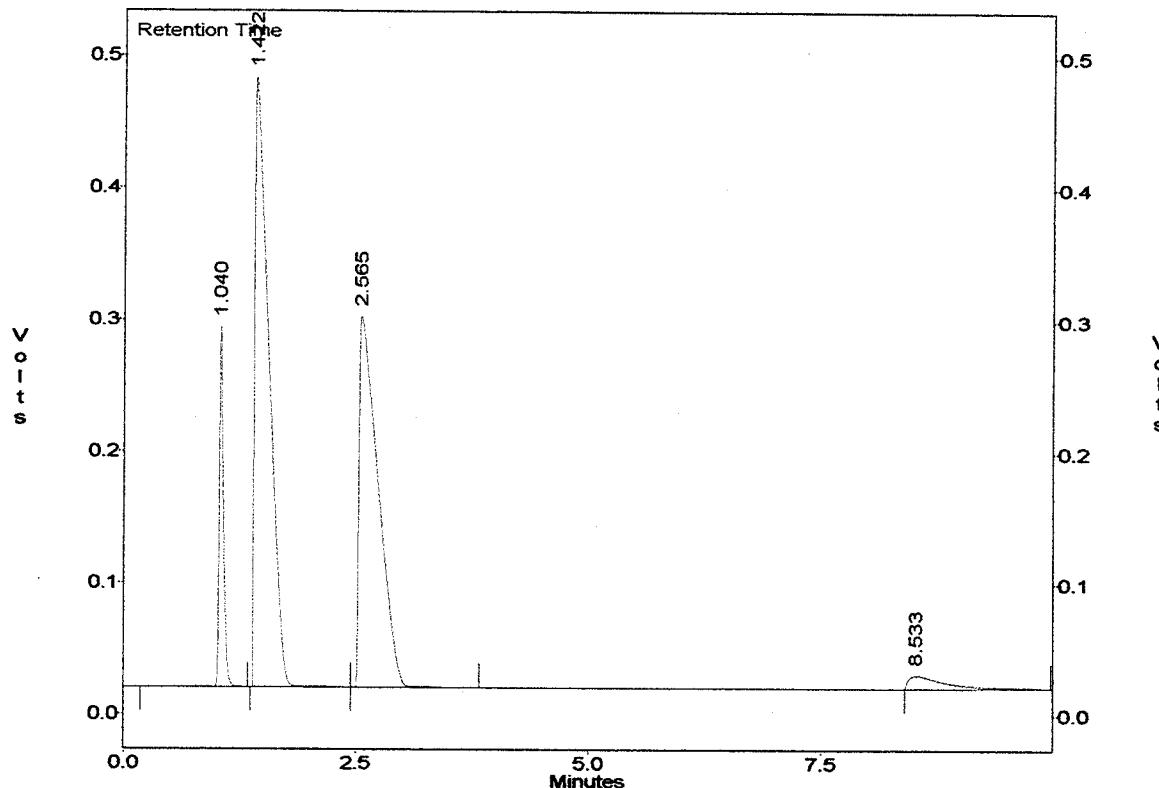
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.435	5506645.0	68.42
3	Carbon Dioxide	2.610	3537088.0	31.58
Totals :			9043733.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.606
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL / 17
Printed : Jun 06, 1998 23:25:15

C:\LABQUEST\CHROM\ROD.606 -- Channel A



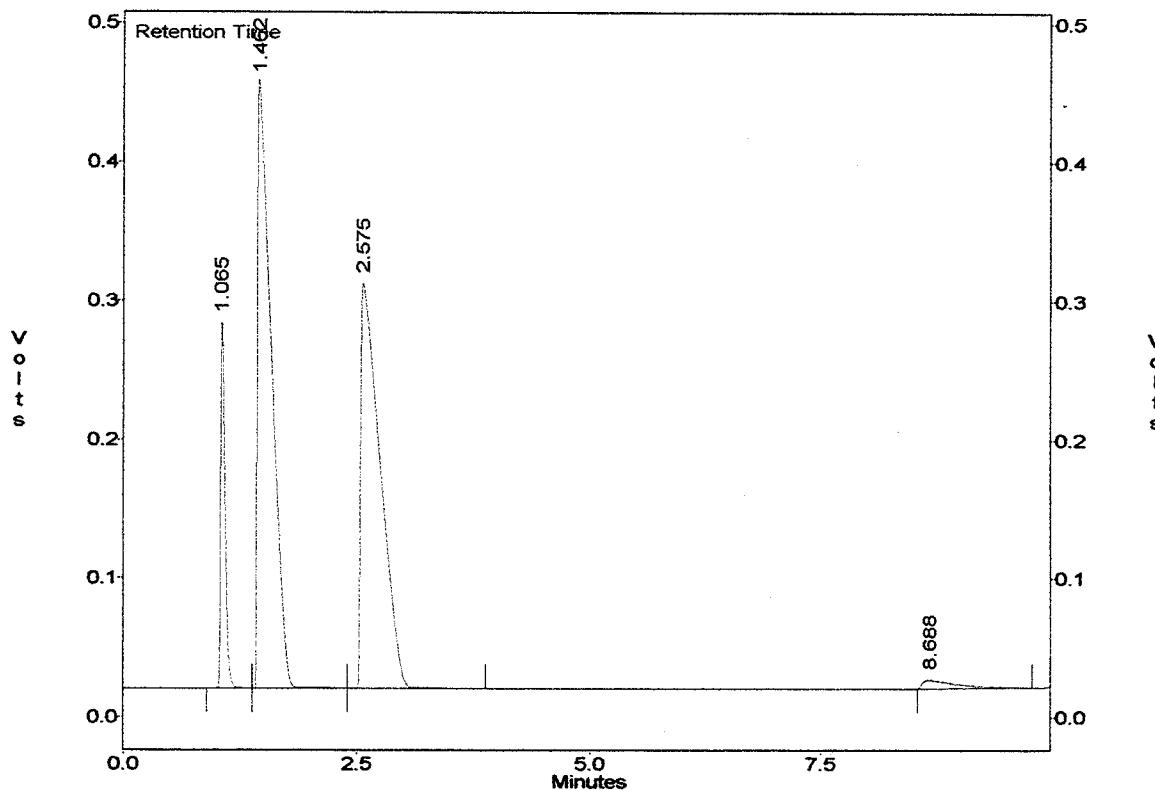
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.422	4875957.0	63.00
3	Carbon Dioxide	2.565	3986138.3	37.00
Totals :			8862095.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.606
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL / 14
Printed : Jun 06, 1998 13:58:58

C:\LABQUEST\CHROM\ROD.606 - Channel A



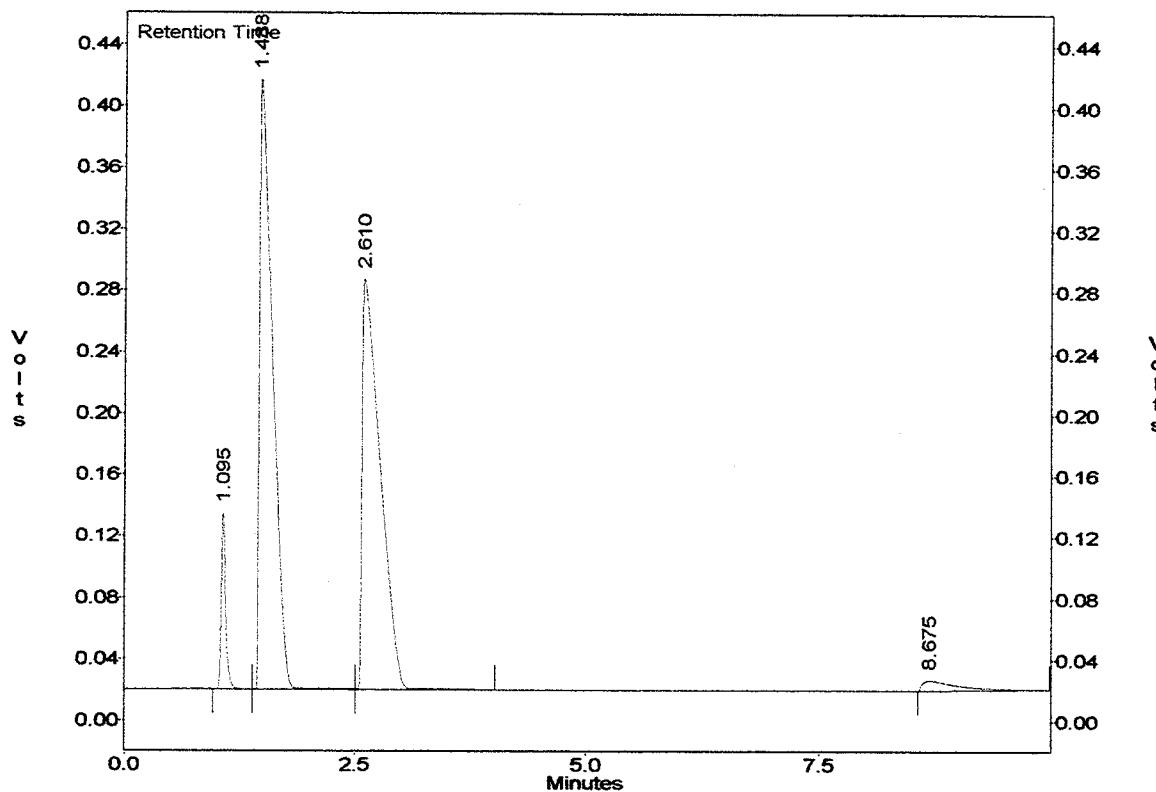
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.462	4437186.0	59.35
3	Carbon Dioxide	2.575	4230029.0	40.65
Totals :			8667215.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.606
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL / 11
Printed : Jun 06, 1998 13:42:28

C:\LABQUEST\CHROM\ROD.606 -- Channel A



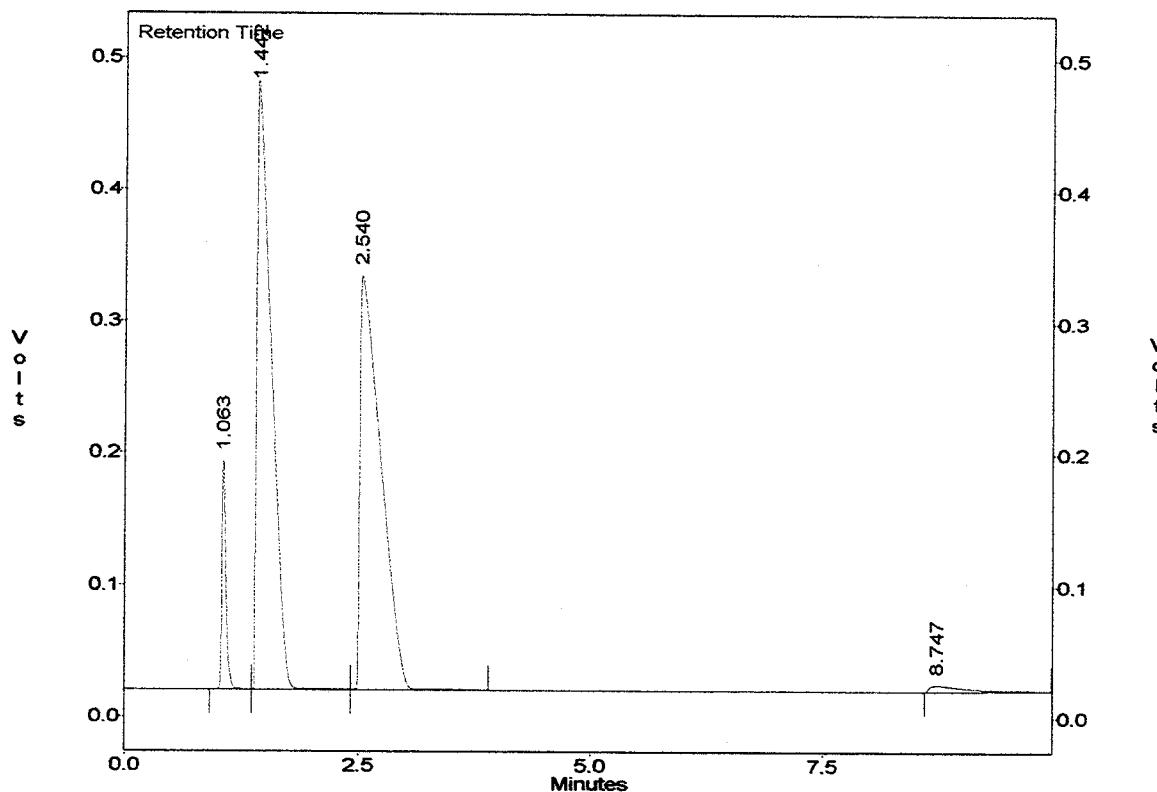
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.488	3759369.8	59.01
3	Carbon Dioxide	2.610	3633971.0	40.99
Totals :			7393341.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.606
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL / 8
Printed : Jun 06, 1998 13:21:33

C:\LABQUEST\CHROM\ROD.606 -- Channel A

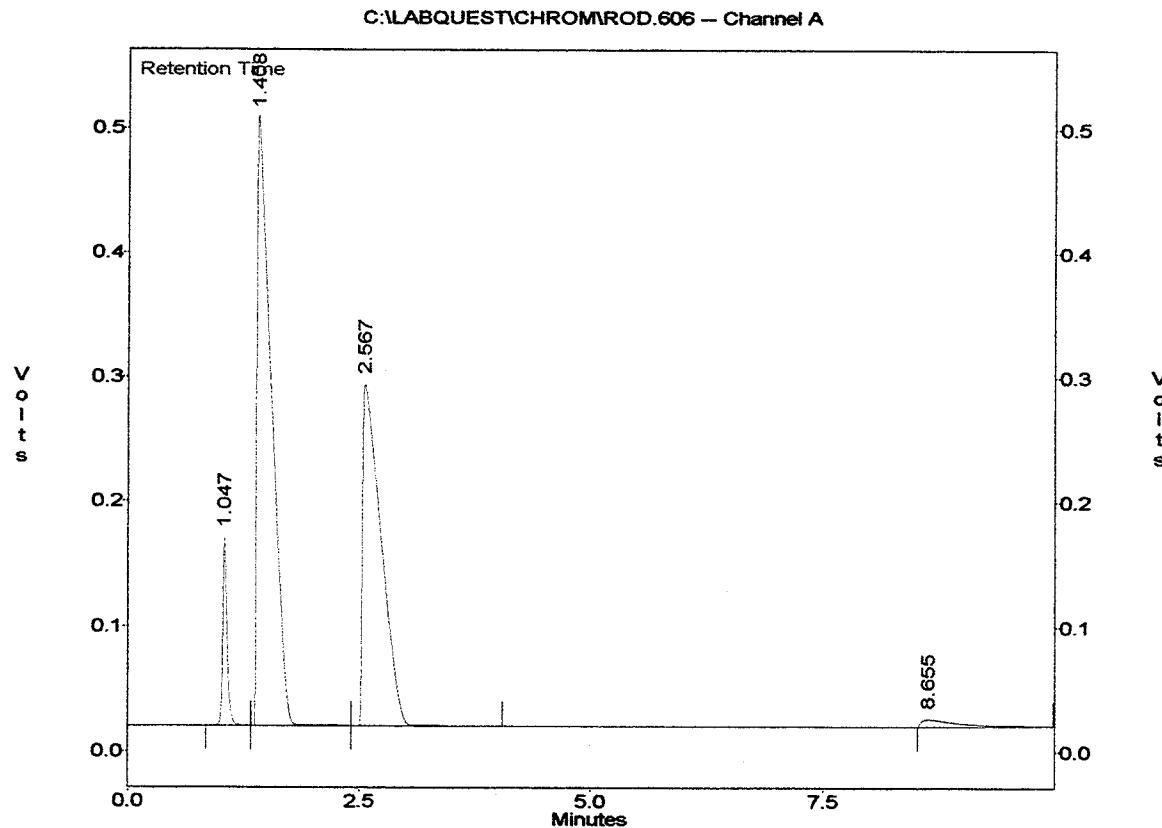


Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.442	4820590.5	58.50
3	Carbon Dioxide	2.540	4760156.5	41.50
Totals :			9580747.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.606
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL / 5
Printed : Jun 06, 1998 13:05:53

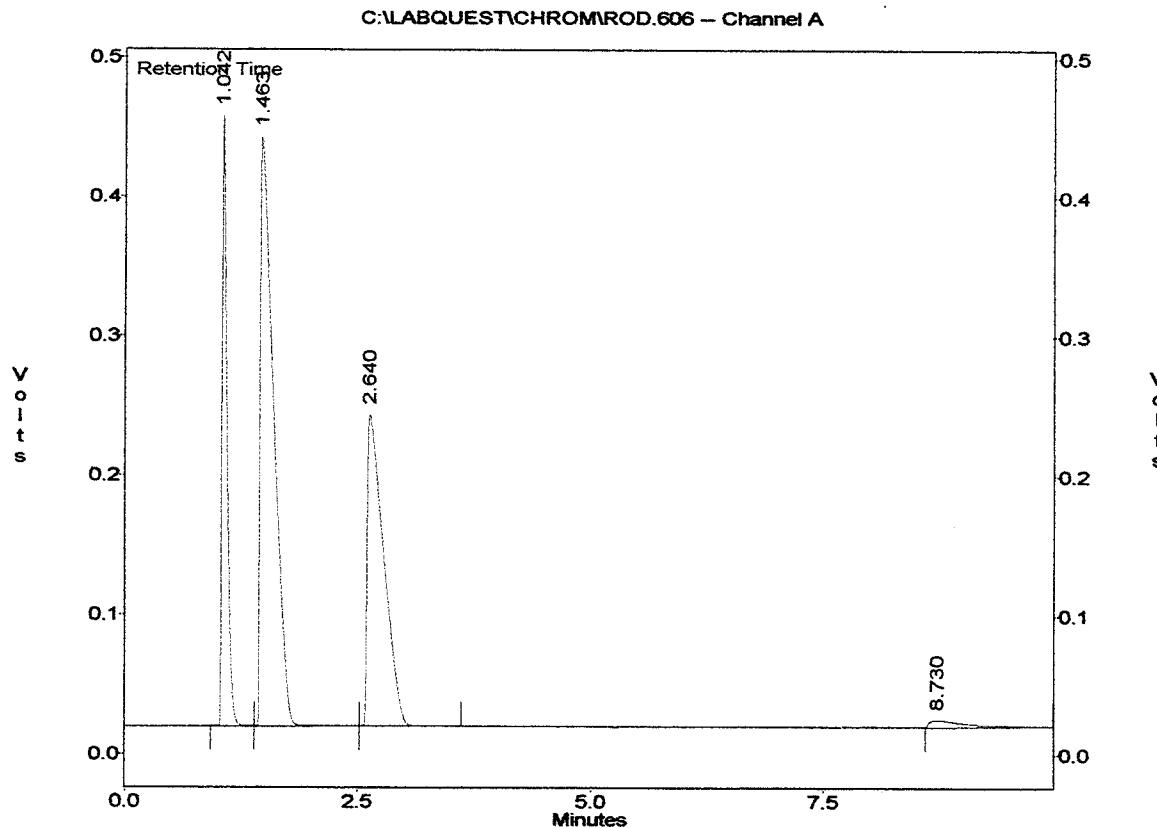


Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.408	5293552.5	66.13
3	Carbon Dioxide	2.567	3772505.0	33.87
Totals :			9066058.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\ROD.606
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL / 2
Printed : Jun 06, 1998 12:47:11



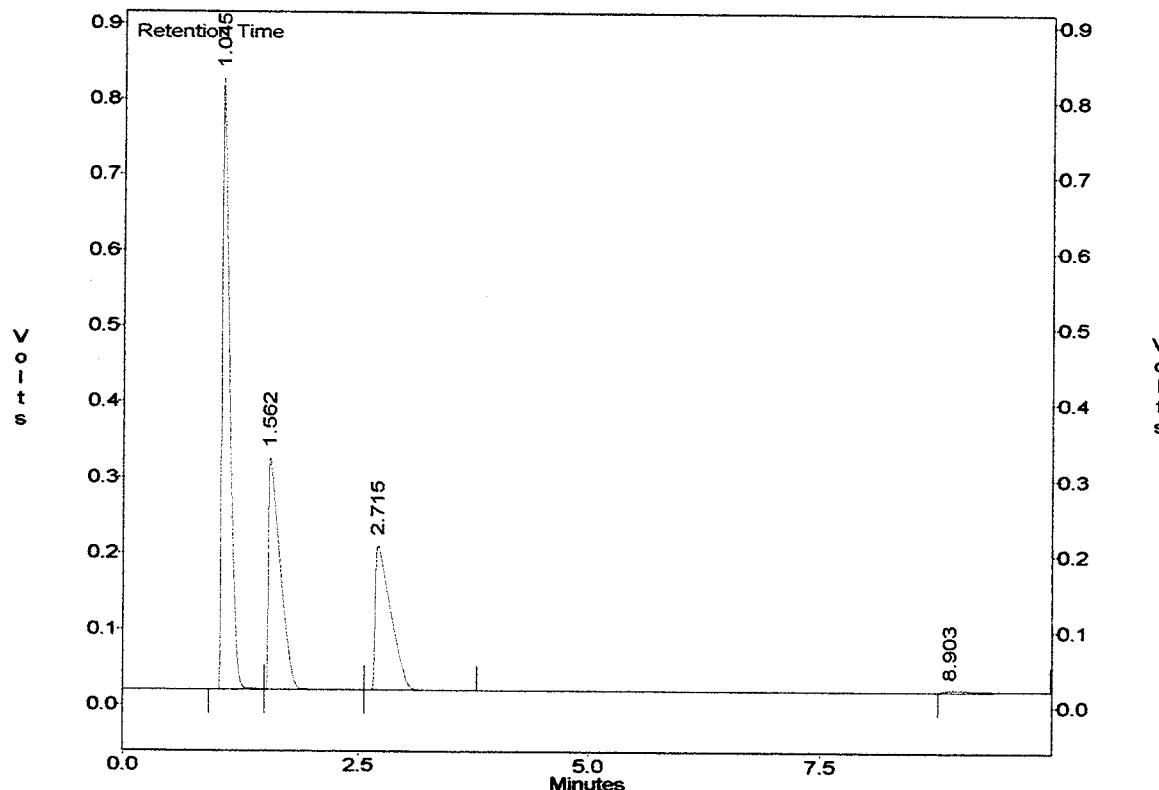
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.463	4211825.0	68.16
3	Carbon Dioxide	2.640	2738296.5	31.84
Totals :			6950121.5	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\CJR.529
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL #1
Printed : May 29, 1998 19:03:47

C:\LABQUEST\CHROM\CJR.529 - Channel A



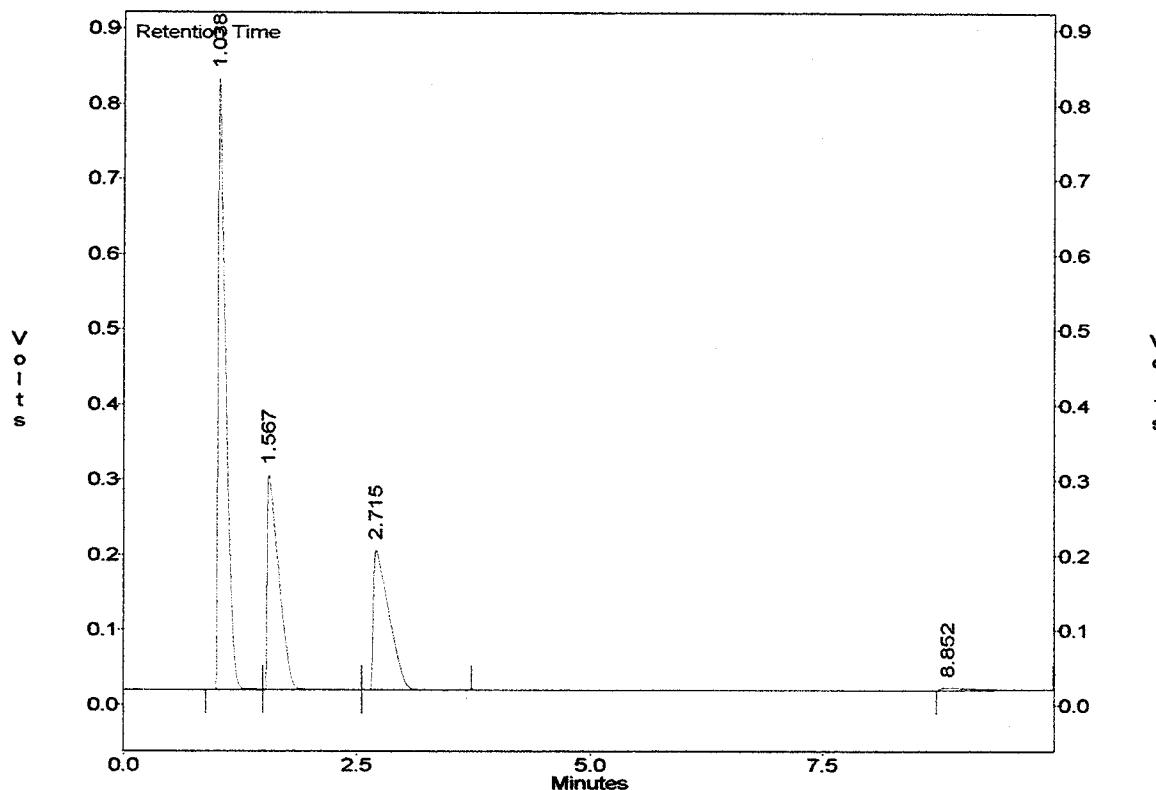
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.562	2638135.8	62.41
3	Carbon Dioxide	2.715	2211822.8	37.59
Totals :			4849958.5	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\CJR.529
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL #4
Printed : May 29, 1998 19:31:11

C:\LABQUEST\CHROM\CJR.529 - Channel A



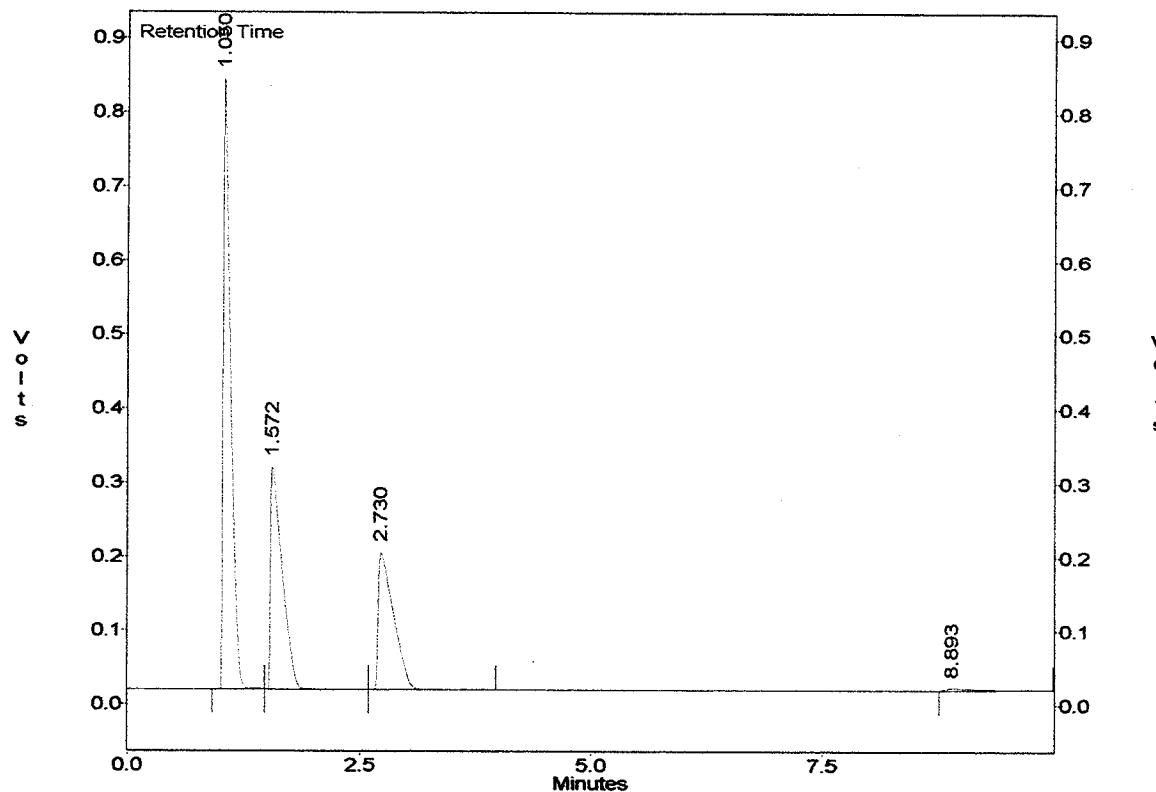
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.567	2387867.3	60.99
3	Carbon Dioxide	2.715	2125146.0	39.01
Totals :			4513013.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\CJR.529
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL #7
Printed : May 29, 1998 20:07:53

C:\LABQUEST\CHROM\CJR.529 - Channel A



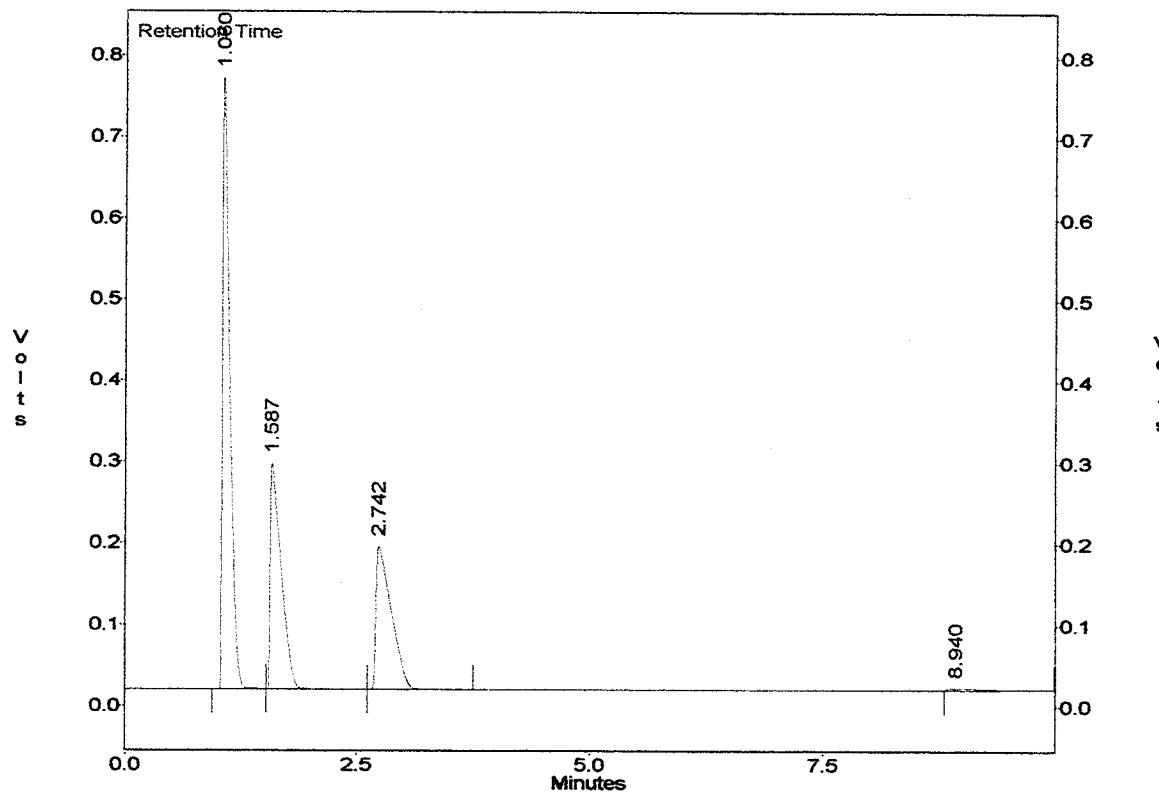
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.572	2584103.3	62.76
3	Carbon Dioxide	2.730	2133925.0	37.24
Totals :			4718028.0	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\CJR.529
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL #10
Printed : May 29, 1998 20:30:15

C:\LABQUEST\CHROM\CJR.529 -- Channel A



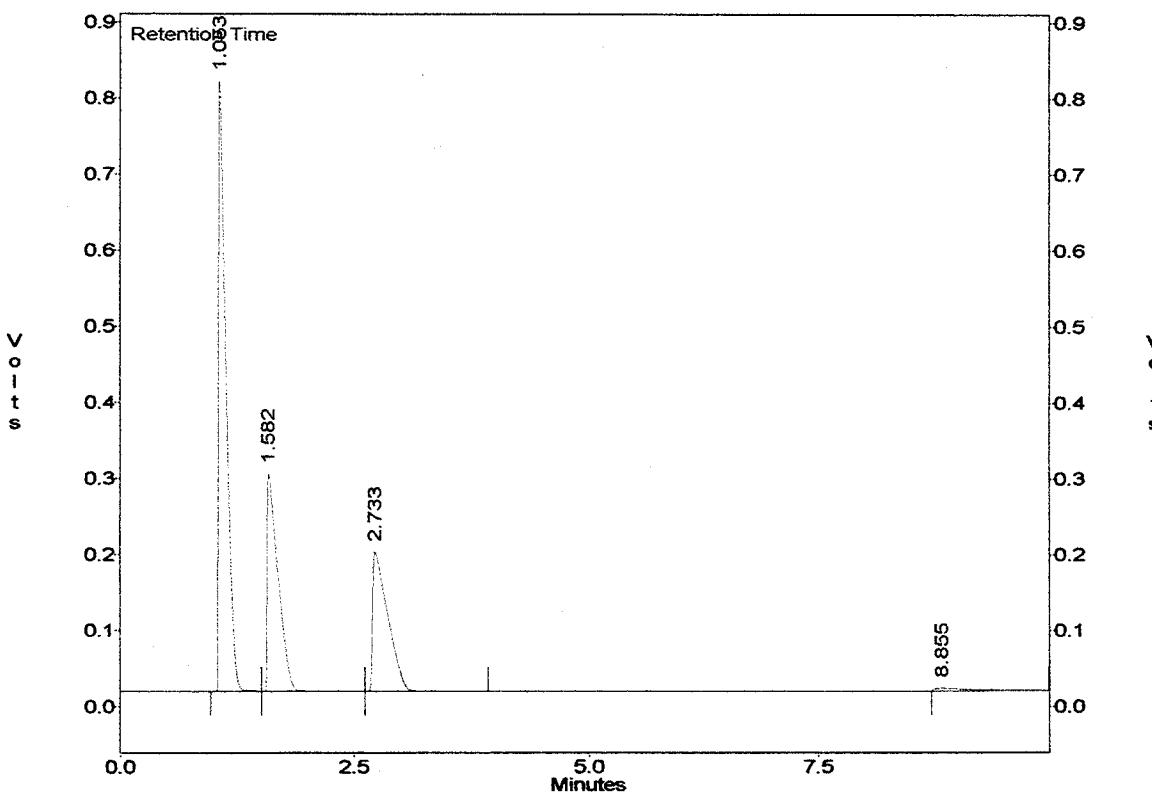
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.587	2262604.5	61.85
3	Carbon Dioxide	2.742	1942272.1	38.15
Totals :			4204876.5	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\CJR.529
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL #13
Printed : May 29, 1998 20:46:19

C:\LABQUEST\CHROM\CJR.529 - Channel A



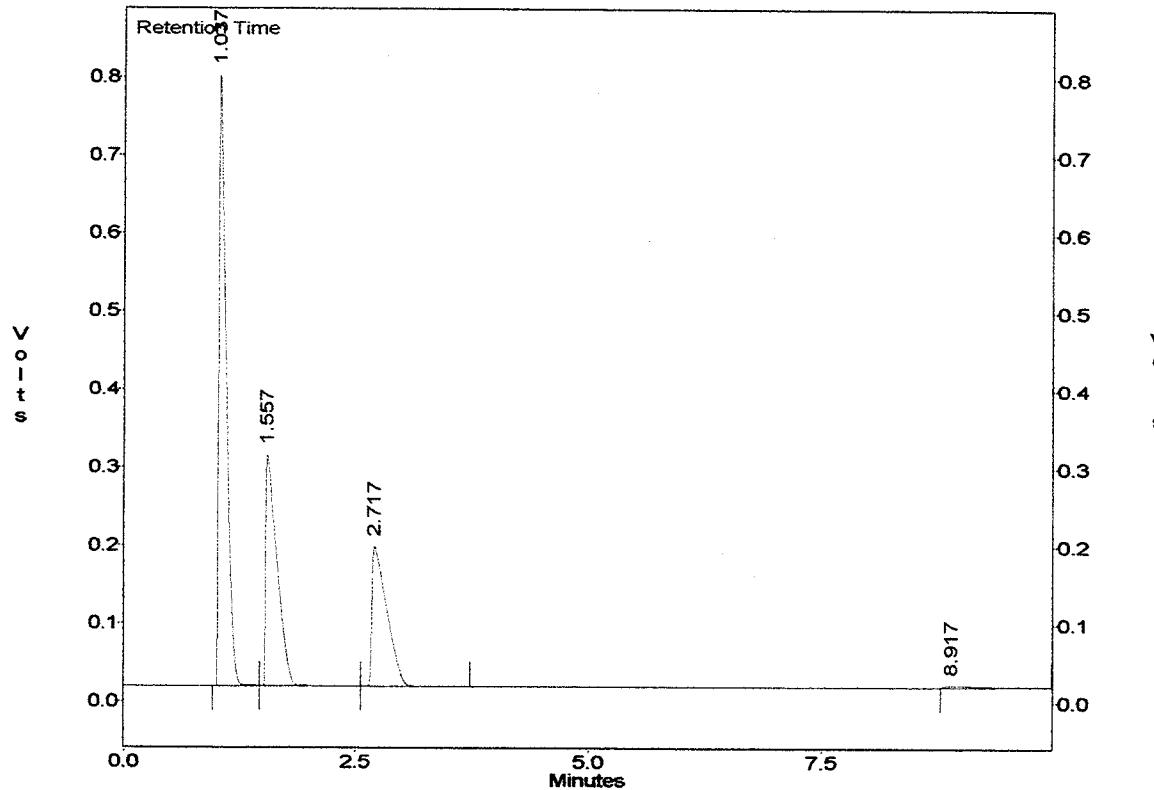
Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.582	2361568.3	61.30
3	Carbon Dioxide	2.733	2075262.1	38.70
Totals :			4436830.5	100.00

Gas Analysis

File : C:\LABQUEST\CHROM\CJR.529
Method : C:\LABQUEST\METHODS\biogas.MET
Sample ID : NREL #16
Printed : May 29, 1998 21:08:24

C:\LABQUEST\CHROM\CJR.529 - Channel A



Channel A Results

Peak Number	Peak Name	Retention Time	Area	Norm Conc
2	Methane	1.557	2523193.3	63.47
3	Carbon Dioxide	2.717	2021501.1	36.53
Totals :			4544694.5	100.00

Calculated Data

Other Analysis

LABORATORY SOLIDS WORKSHEET

Time	2355									
Date	5/10/95									
Sample I.D.	MTX 7F 6P									
Crucible I.D.	1	2	3	4	5	6				
Weights	#	Equation								
Crucible	1		1.096	1.102	1.103	1.105				
Wet Sample + Crucible	2									
Wet Sample	3	2 - 1	2.923	2.912	2.892	2.891				
Dry Sample + Crucible	4									
Dry Sample	5	4 - 1	.140	.125	.127	.084				
Ash Sample + Crucible	6		1.113	1.113	1.115	1.117				
Ash Sample	7	6 - 1	.017	.011	.012	.012				
Volatile Solids	8	5 - 7	.123	.114	.115	.072				
ANALYSIS										
% Total Solids		(5 / 3) x 100	4.79	4.29	4.41	4.49	2.91	2.68	2.61	2.73
% Volatile Solids		(8 / 5) x 100	87.85	91.20	90.55	89.86	85.71	88.46	88.15	87.44

LABORATORY SOLIDS WORKSHEET

Time	0320							
Date	5/7/98							
Sample I.D.	T. O ₂							
Crucible I.D.		1	2	3				
WEIGHTS	#	Equation						
Crucible	1		.108	.097	.100			
Wet Sample + Crucible	2							
Wet Sample	3	2 - 1	.922	.940	.975			
Dry Sample + Crucible	4							
Dry Sample	5	4 - 1	.169	.153	.170			
Ash Sample + Crucible	6		.147	.143	.142			
Ash Sample	7	6 - 1	.039	.046	.042			
Volatile Solids	8	5 - 7	.130	.107	.128			
ANALYSIS								
% Total Solids		(5 / 3) x 100	5.78	5.20	5.71	5.54		
% Volatile Solids		(8 / 5) x 100	76.92	69.93	75.29	74.04		

LABORATORY SOLIDS WORKSHEET

	Time	2350					
C	Date	5/11/98					
	Sample I.D.	HYDROLY 3A					
	Crucible I.D.	CENTRE L	1	2	3		
Wt. GRMS	#	Equation					
Crucible	1		1.103	1.105	1.110		
Wet Sample + Crucible	2						
Wet Sample	3	2 - 1	2.901	2.901	2.904		
Dry Sample + Crucible	4						
Dry Sample	5	4 - 1	.151	.151	.151		
Ash Sample + Crucible	6		1.129	1.132	1.143		
Ash Sample	7	6 - 1	.024	.024	.027		
Volatile Solids	8	5 - 7	.125	.124	.124		
ANALYSIS							
% Total Solids		(5 / 3) x 100	52.1	52.1	52.0	52.0	
% Volatile Solids		(8 / 5) x 100	82.78	82.11	82.11	82.33	

LABORATORY SOLIDS WORKSHEET

Time		5-30-98						
Date		Noon						
Sample I.D.		Bacto Peptone						
Crucible I.D.		1 2 3						
WEIGHTS	#	Equation						
Crucible	1		0.991	0.984	1.024			
Wet Sample + Crucible	2							
Wet Sample	3	2 - 1						
Dry Sample + Crucible	4							
Dry Sample	5	4 - 1	0.170	0.120	0.101			
Ash Sample + Crucible	6		0.999	0.988	1.029			
Ash Sample	7	6 - 1	0.008	0.004	0.005			
Volatile Solids	8	5 - 7	0.162	0.116	0.096			
ANALYSIS								
% Total Solids		(5 / 3) x 100	5.81	6.10	5.09			
% Volatile Solids		(8 / 5) x 100	95.29	96.67	95.05			

$$\% TS = 5.67$$

$$\% VS = 95.67$$

Temp	90 C
Mode	Auto
	100.0%
0.0M	2.924 g
5.0M	87.9 %
10.0M	75.9 %
15.0M	62.9 %
20.0M	51.5 %
25.0M	40.6 %
30.0M	30.2 %
35.0M	21.3 %
40.0M	13.1 %
45.0M	6.7 %
Time off.	47.5Min
	5.81 %
	0.170 g

Temp	90 C
Mode	Auto
	100.0%
0.0M	1.966 g
5.0M	88.5 %
10.0M	76.3 %
15.0M	64.8 %
20.0M	54.1 %
25.0M	43.9 %
30.0M	34.3 %
35.0M	25.2 %
40.0M	17.0 %
45.0M	10.3 %
50.0M	6.3 %

Time off.	59.5Min
	6.10 %
	0.120 g

LABORATORY SOLIDS WORKSHEET

Time		5:30 pm					
Date		5-30-98					
Sample I.D.		Terminal Island Sewage Sludge					
Crucible I.D.		1	2	3			
WEIGHTS	#	Equation					
Crucible	1		0.983	0.988			
Wet Sample + Crucible	2						
Wet Sample	3	2 - 1					
Dry Sample + Crucible	4						
Dry Sample	5	4 - 1	0.067	0.064			
Ash Sample + Crucible	6		1.006	1.014			
Ash Sample	7	6 - 1	0.023	0.026			
Volatile Solids	8	5 - 7	0.044	0.038			
ANALYSIS							
% Total Solids		(5 / 3) x 100	3.20	3.01			
% Volatile Solids		(8 / 5) x 100	65.67	59.38			

$$\% TS = 3.105$$

$$\% VS = 62.53$$

Temp Mode	90 C Auto 100..0%
0.0M	2.094 g
5.0M	89.5 %
10.0M	76.4 %
15.0M	63.7 %
20.0M	51.6 %
25.0M	39.6 %
30.0M	28.3 %
35.0M	18.2 %
40.0M	10.1 %
45.0M	5.1 %
Time eff.	49.0Min 3.20 % 0.067 g
Temp Mode	90 C Auto 100..0%
0.0M	2.128 g
5.0M	85.3 %
10.0M	69.9 %
15.0M	55.2 %
20.0M	40.7 %
25.0M	27.0 %
30.0M	15.1 %
35.0M	7.7 %
40.0M	4.0 %
Time eff.	43.0Min 3.01 % 0.064 g

5:30pm

0.5 ml into 9.5 ml water

1:20

Actual Conc mg/L

	Bkg	0	0	
[]	STD 0.5	45	45	450
[]	STD 1.0	99	97	970
[]	Pep 1	1650	277	55,400
[]	Pep 2	1650	291	58,200
[]	Pep 3	1650	293	58,600
[]	7F 1	1650	172	34,400
[]	7F 2	1650	174	34,800
[]	7F 3	1650	176	35,200
[]	TiO ₂ 1	1650	271 TiO ₂ 1	54,200
[]	BF 1	1650	282 TiO ₂ 2	56,400
[]	3A 1	1650	284 TiO ₂ 3	56,800

Terminal Island Anaerobic digester Sludge

[]	pH = 7.43	BF 1	140	28,000
[]		BF 2	141	28,200
[]		BF 3	143	28,600
[]		3A 1	267	53,400
[]		3A 2	275	55,000
[]		3A 3	274	54,400
[]				54,800

Final PH

- 1 7.25
- 2 7.28
- 3 7.27
- 4 7.36
- 5 7.37
- 6 7.36
- 7 7.23
- 8 7.24
- 9 7.25
- 10 7.22
- 11 7.22
- 12 7.23
- 13 7.23
- 14 7.23
- 15 7.24
- 16 7.23
- 17 7.23
- 18 7.25

COD RESULTS

Secondary (X 1000)

- #1 - Control Hydrolyzate
 - 1- 63
 - 2- 53
 - 3- 51
 - Avg - 55.66

Primary CR

54,600

#2 - BF 772014

- 1- 27
- 2- 26
- 3- 26
- Avg - 26.33

28, 267

#3 - MTX 7F BROTH

- 1- 31
- 2- 34
- 3- 32
- Avg - 32.33

34, 800

#4 - Ti O₂ TREATED

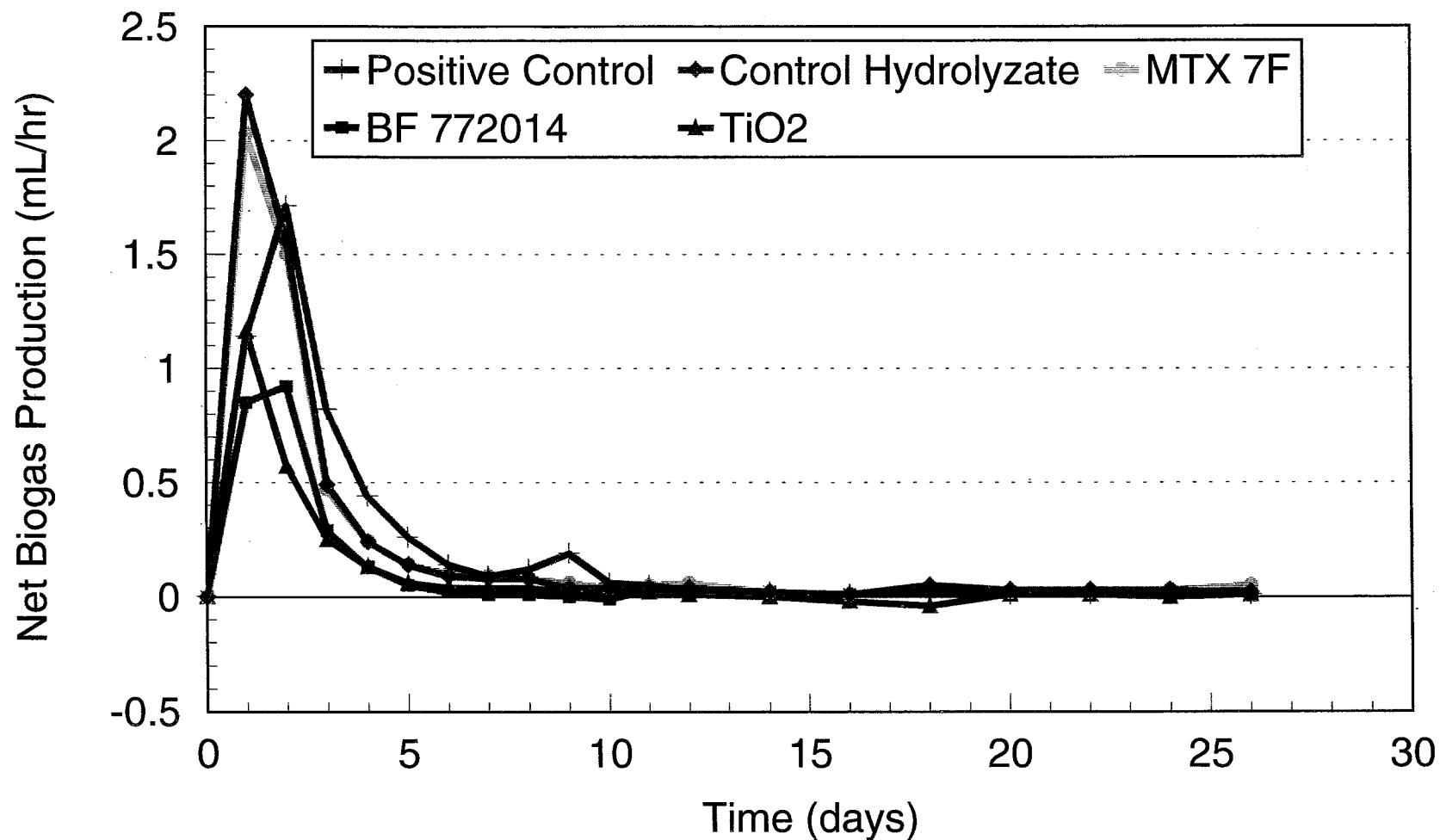
- 1- 50
- 2- 58
- 3- 52
- Avg - 53.33

55, 800

Graphics

Biochemical Methane Potential Assay

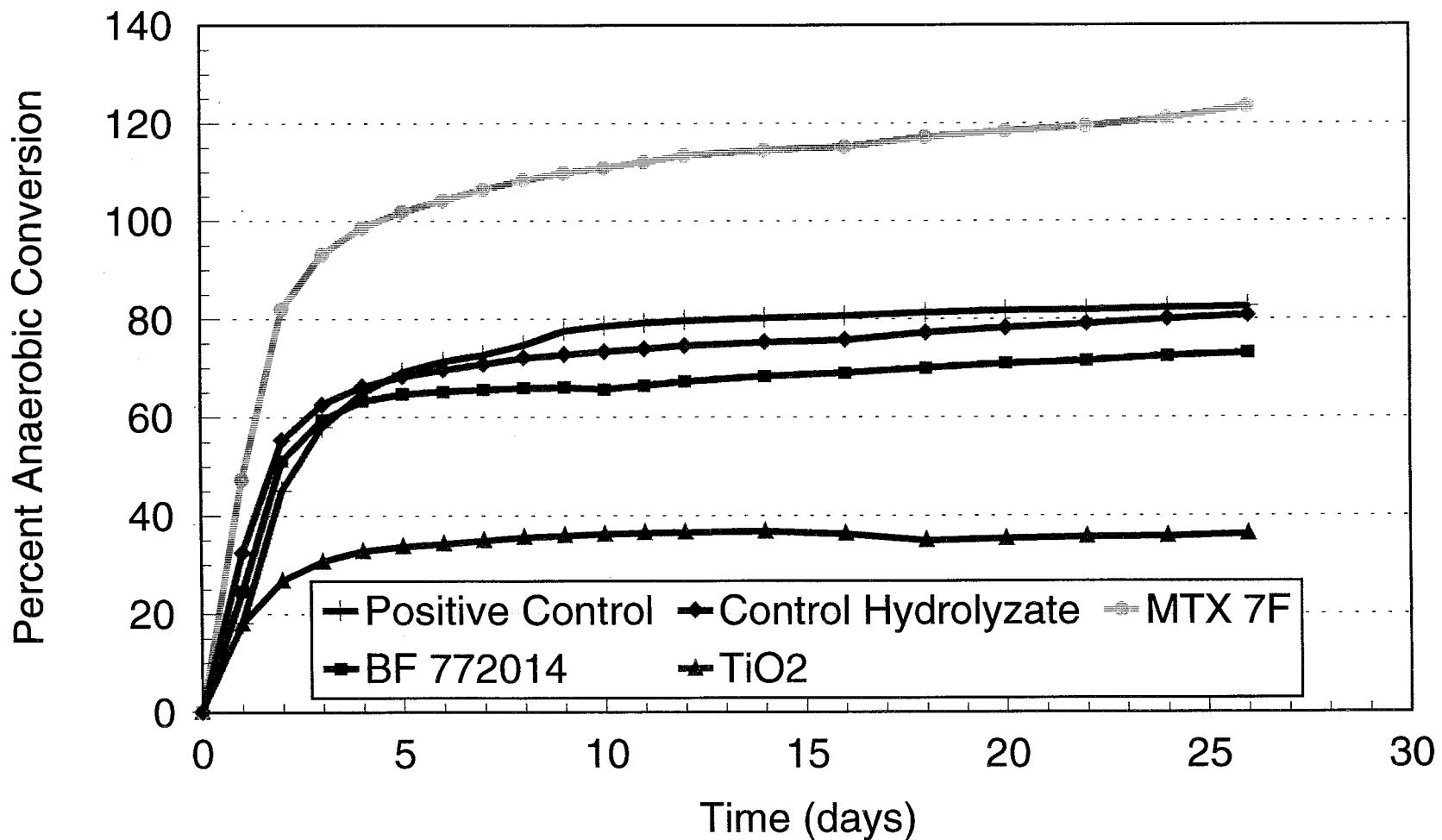
NREL Ethanol Process Samples



Pinnacle Biotechnologies International, 1998

Biochemical Methane Potential Assay

NREL Ethanol Process Samples



Pinnacle Biotechnologies International, 1998

-----Original Message-----

From: Brian Duff [SMTP:pinnaclebiotech@worldnet.att.net]
Sent: Wednesday, October 18, 2000 1:39 PM
To: Nick Nagle
Subject: BMP data for ethanol database

Hi Nick:

Nice speaking with you again.

I have no problem with you including the BMP work that Pinnacle performed for NREL on ethanol process residues back in 1998.

Thank you for checking first, I appreciate being in the loop.

Talk to you soon, Brian Duff